Generic Aspects of Molluscan Shell Morphogenesis:

Theoretical, Experimental and Comparative Approaches

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Séverine Urdy

aus

Frankreich

Promotionskomitee

Prof. Dr. Hugo Bucher
(Leiter der Dissertation, Vertreter der Universität Zürich)
Dr. Régis Chirat
Prof. Dr. Øyvind Hammer
Prof. Dr. Marcelo R. Sánchez-Villagra
Prof. Dr. Christoph P. E. Zollikofer

Zürich, 2008

Contents

GENERIC ASPECTS OF MOLLUSCAN SHELL MORPHOGENESIS:

THEORETICAL, EXPERIMENTAL AND COMPARATIVE APPROACHES

By

Séverine Urdy

Ph. D Thesis – 2008

Abstract for non-academic audience

Molluscs are well suited to address the relationships between evolution and development. First, molluscs have an excellent fossil record. Second, due to accretionary growth, each single shell records the shape changes that occurred during the growth of the animal. Third, shells have a simple geometry which often conforms to a logarithmic spiral thus facilitating theoretical investigations. Studies comparing ammonoids and gastropods suggest that common rules underlie the morphogenesis of the shell and its evolution in both clades. The objective of this thesis is to investigate these rules theoretically and experimentally. In a population of recent marine gastropods raised in controlled laboratory conditions, the patterns of covariation between shell features appear similar to that reported for some ammonoids. They can be explained by a simple growth model, simulating accretionary growth.

Zusammenfassung für nichtakademisches Laienpublikum

Mollusken sind ausgezeichnet geeignet um die Zusammenhänge zwischen Evolution und Entwicklung zu untersuchen. Erstens haben die Mollusken einen hervorragenden Fossilbericht, zweitens zeichnen die Mollusken wegen ihres akkretionären Wachstums Formveränderungen während ihrer Ontogenie auf, und drittens haben deren Schalen eine einfache Geometrie welche oft einer logarithmischen Spirale entspricht und deswegen theoretische Untersuchungen erleichtert. Die Ergebnisse vergleichender Studien an Ammonoideen und Gastropoden legen nahe, dass die Morphogenese und Evolution der Schalen beider Clades gemeinsamen Regeln zugrunde liegen. Das Ziel der vorliegenden Arbeit ist diese Regeln sowohl theoretisch als auch experimentell zu untersuchen. In einer Population rezenter mariner Gastropoden welche unter kontrollierten Laborbedingungen aufgezogen wurden, erscheinen die Muster der Kovariation zwischen verschiedenen Schalenmerkmalen ähnlich wie bei manchen Ammonoideen. Dies lässt sich mit einem einfachen Wachstumsmodell erklären, welches akkretionäres Wachstum simuliert.

ABSTRACT

How an embryo develops its particular form during ontogeny and how shape changes through evolutionary time are two closely linked questions. An approach to these issues, mainly inspired by D'Arcy Thompson's work, is to highlight the 'laws of form', that is how developmental systems determine the variation of organismal forms on short and long time scales. During the last decades, theoretical models of morphogenesis have allowed the identification of some of these rules from experimentally well-studied developmental systems.

Molluscs are well suited to address the relationships between evolution and development. The preservation of the ontogeny of the shell due to its accretionary growth and the excellent fossil record in this group are undeniable advantages. Also, molluscan shell shape and growth have been the focus of extensive theoretical work, revealing the regularity of accretionary growth which often conforms well to logarithmic spiral coiling.

The study of evolutionary changes occurring in mollusc lineages relies nearly exclusively on the interpretation of shell morphologies. Important taxonomic features of molluscs include the shape of the aperture, the degree of coiling of the shell tube, the ornamentation (ribs, tubercles, spines, keels) and growth features (growth halts, constrictions, varices). The evolution of the molluscan shell is characterized by frequent convergences in form and ornamentation. As a consequence, the recognition of transformation of one shape into another crucially depends on the knowledge of how these shell shapes are generated.

The comparison between different clades of molluscs can be informative with regards to the basic rules of accretionary growth. In particular, it has been pointed out that common rules of accretionary growth could underlie the morphogenesis of the shell and its evolution in ammonoids and gastropods. Evidences come from the comparison of intraspecific patterns of covariation between shell characters, from the examination of growth changes occurring at maturity and from the analysis of teratological shells with malformations caused by injuries or change in living conditions in both clades.

In some highly variable ammonoids species, it has been shown that simple growth rules could underlie the evolutionary recurrent covariation of aperture shape, degree of coiling and intensity of the ornamentation (Buckman's law of covariation). Similarly, these characters covary with the spacing between growth halts during the ontogeny of some ammonoids species.

A central objective of this thesis is to investigate what kinds of generic rules could produce the patterns of variation of molluscan shell shape. In a first part, it is discussed how generic models can inform us about the generation and evolution of structures of particular size and shape. In a second part, a null hypothesis model of shell growth is proposed. The intricate relationships between growth rate and allometry are described. The kind of morphological variation expected given these basic growth rules is compared to experimental evidence in developmentally plastic shells of intertidal gastropods. A population of recent gastropods (*Hexaplex trunculus*, Muricidae), originated from a single egg mass and bred in laboratory for about a year and a half, is used to describe the ontogenetic patterns of covariation between shell characters and the dynamics of growth. This study highlights

a covariation between growth rhythm (frequency and amplitude of pulses of growth), growth halts spacing, aperture allometry and intensity of ornamentation. In particular, variation in growth rhythm is regarded as critical in generating the observed covariation between growth halts spacing and ornamentation. A simple growth model is proposed to account for the covariation of these shell characters. Some recurrent patterns of variation in ammonoids species could result from similar rules tied to basic constraints of accretionary growth.

The theoretical and empirical framework developed here can assist in formulating and testing new hypotheses of growth of molluscan shells. It paves the way toward the development of data-driven mathematical models which could facilitate the comparison of theoretical and empirical data in the future, and perhaps helps interpreting them in a developmental, ecological and evolutionary context. More generally, this dissertation argues that the time parameter is mandatory to the study of allometry, if one seeks to understand the relationships between size and shape and how they vary in populations.

Key words: molluscs - growth - generic morphogenetic models - allometry - morphometry - variation - plasticity - growth halts - ontogeny - evolution - structuralism.

ZUSAMMENFASSUNG

Wie ein Embryo seine Form im Laufe der Ontogenie entwickelt und wie sich die Form im Laufe der Evolution ändert sind zwei eng miteinander verbundene Fragen. Die Arbeit von D›Arcy Thompson liefert einen wichtigen Ansatz zu deren Lösung; dementsprechend wurde hier ein Schwerpunkt auf die "laws of form" (Gesetze der Form) gelegt, d.h. wie ein sich entwickelndes System die Variation organismischer Formen über kurze oder lange Zeiträume hinweg bestimmen kann. Theoretische Modelle der Morphogenese, wie sie im Verlaufe der letzen Jarhrzehnte entwickelt wurden, haben die Aufstellung von Regeln gestützt auf Experimente mit gut untersuchten Entwicklungssystemen erlaubt.

Mollusken sind gut geeignet, um die Zusammenhänge zwischen Evolution und Entwicklung zu ergründen. Sowohl die Überlieferung der Schalen-Ontogenie wegen ihres akkretionären Wachstums als auch der exzellente Fossilbericht sind zweifelsohne von grossem Vorteil. Weiterhin standen Form und Wachstum der Molluskenschalen im Mittelpunkt umfangreicher theoretischer Studien, die die Regelmässigkeit akkretionären Wachstums aufzeigten, welche oft recht gut einer logarithmisch-spiraligen Aufrollung entspricht.

Üblicherweise basieren Studien evolutionärer Veränderungen in Mollusken-Stammeslinien fast ausschliesslich auf der Interpretation der Schalenmorphologie. Taxonomisch bedeutsame Merkmale der Mollusken umfassen die Form der Mündung, der Aufrollungsgrad der Schalenröhre, die Ornamentierung (Rippen, Tuberkel, Stacheln, Kiele) und Wachstumsaspekte (Wachstumsunterbrechungen, Einschnürungen, Varices). Häufige Konvergenzen in Form und Ornamentierung sind kennzeichend für die Evolution der Molluskenschale. Folglich hängt die Erkennung der Umwandlung von einer in eine andere Form grundlegend von der Kenntnis ab, wie diese Schalenformen gebildet wurden.

Der Vergleich zwischen verschieden Mollusken-Kladen kann wertvolle Informationen liefern hinsichtlich der Grundregeln akkretionären Wachstums. Im Besonderen wurde darauf hingewiesen, dass die Schalen-Morphogenese von Ammonoideen und Gastropoden gemeinsamen Regeln akkretionären Wachstums zugrunde liegen. Dies lässt sich zeigen durch Vergleiche der Muster der intraspezifischen Kovariation von Schalenmerkmalen, durch die Untersuchung von Reife-bedingten Wachstumsveränderungen sowie durch die Analyse teratologischer Schalen mit Missbildungen durch Verletzungen oder Veränderungen in den Wachstumsbedingungen in beiden Kladen.

Für manche hochgradig variable Ammonoideen-Arten wurde gezeigt, dass einfache Wachstumsregeln der evolutionär sich wiederholenden Kovariation von Mündungsform, Aufrollungsgrad und Stärke der Ornamentierung (Buckman's law of covariation) zugrunde liegen. In ähnlicher Weise kovariierien diese Merkmale mit dem Abstand zwischen Wachstumsunterbrechungen in der Ontogenie mancher Ammonoideen.

Ein zentrales Anliegen der vorliegenden Arbeit ist es zu untersuchen, auf welchen allgemeinen Regeln die Muster der Variation den Mollusken-Schalenform basieren. Im ersten Teil wird erörtert wie diese allgemeinen Modelle uns Informationen über die Bildung und Evolution von Strukturen bestimmter Grösse und Form liefern können. Im zweiten Teil wird eine Null-Hypothese für

das Schalenswachstum vorgeschlagen. Die komplexen Zusammenhänge zwischen Wachstumsraten und Allometrie werden beschrieben. Die auf den allgemeinen Regeln basierenden Vorhersagen zur morphologische Variation werden mit den experimentellen Beweisen der Entwicklungs-Plastizität der Schalen intertidaler Gastropoden verglichen. Ontogenetische Muster der Kovariation zwischen Schalenmerkmalen und Wachstumsdynamik werden anhand einer Population rezenter Gastropoden (Hexaplex trunculus, Muricidae) beschrieben, welche aus einem einzigen Eigelege im Labor über eineinhalb Jahre aufgezogen wurde. Diese Untersuchung hebt eine Kovariation von Wachstumsrhythmen (Häufigkeit und Amplitude von Wachstumspulsen), Abstand zwischen Wachstumsunterbrechungen, allometrischen Veränderungen der Mündungsform und Stärke der Ornamentierung hervor. Besonders die Variation der Wachstumsrhythmen wird als entscheidender Faktor für die Generierung der beobachteten Kovariation zwischen Wachstumsunterbrechungen und Ornamentierung angesehen. Ein einfaches Wachstumsmodell wird vorgestellt welches die Kovariation dieser Schalenmerkmale berücksichtigt. Einige wiederkehrende Muster der Variation von Ammonoideenarten könnten von ähnlichen Regeln gesteuert werden, welche von grundlegenden Einschränkungen akkretionären Wachstums abhängen.

Der hier vorgestellte theoretische und empirische Rahmen kann dazu beitragen, neue Hypothesen zum Wachstum von Molluskenschalen zu formulieren und zu testen. Er bereitet den Weg in Richtung der Entwicklung von Daten-basierten mathematischen Modellen welche den Vergleich theoretischer und empirischer Daten sowie deren Interpretation im ontogenetischen, ökologischen und evolutionären Zusammenhang in der Zukunft erleichtern werden. Etwas allgemeiner formuliert legt diese Dissertation dar, dass der Parameter Zeit massgeblich ist für die Untersuchung der Allometrie wenn versucht wird, die Zusammenhänge zwischen Grösse und Form sowie deren Variation innerhalb von Populationen zu verstehen.

Schlüsselwörter: Mollusken - Wachstum - allgemeine morphogenetische Modelle - Allometrie - Morphometrie -Variation - Plastizität - Wachstumsunterbrechungen - Ontogenie - Evolution - Strukturalismus.

INTRODUCTION

Contents

I. General context

How an embryo develops its particular form during ontogeny and how shape changes through evolutionary time are two closely linked questions. These issues have fascinated scientists from different working fields since more than a century but they are still largely unresolved. After nearly half a century of complete separation between embryological and evolutionary studies (Amundson, 2000), the two fields converged in the late 70's-early 80's, opening a trendy avenue of research often referred to as *'Evo-Devo'*.

Research on development has wavered between two antagonistic epistemological positions: *holism* (top-down explanations) and *reductionism* (bottom-up explanations). Holism, the attempt to understand the whole by subrogating the parts, often associated with vitalist influences, used to dominate much of the experimental embryogenesis of the 19th-early 20th century. This epistemological position has been widely discounted from the 40's (e.g. by Schrödinger, 1944 in his book *'What is Life'*). Reductionism, the attempt to reduce the explanation of phenomena to their lower hierarchical levels of organization by assuming a linear chain of causation between these levels (aggregation of parts, atomism), became more and more popular from the 40's. Consequently, *"explanations for biological phenomena were sought in the 'cellular biology' and then increasingly in 'molecular biology'"* (Horder, 2001, p. 111).

However, between the two World Wars,

a middle ground approach has been advocated by several authors, especially by the founders of modern embryology like John Needham, Conrad Waddington, Hans Spemann, Oskar Hertwig, Paul Weiss, etc. Their approach tried to reconcile materialism (by rejecting the vitalism defended by Hans Driesch and Jakob Von Uexküll) with the experimental evidence of emergence (defined below), arguing that it was impossible to develop science wholly from the top-down nor from the bottom-up (Gilbert & Sarkar, 2000). The approach advocated by these embryologists has been variously discussed under the terms *organicism* (Needham, 1930; Von Bertalanffy, 1933, 1952; Gilbert & Sarkar, 2000; El-Hani & Emmeche, 2000; Soto & Sonnenschein, 2005), *structuralism* (Webster & Goodwin, 1982; Ho & Saunders, 1984; Amundson, 2005), *typological thinking* (in opposition to *populational thinking*, e.g. Mayr, 1963; Jenner, 2006) and has been associated with *dialectics* (Waddington, 1947; Levins & Lewontin, 1985; Gilbert & Sarkar, 2000). Modern traditions are variously called *neo-rationalism* or *process structuralism* (Smith, 1992; Resnik, 1994, Griffiths, 1996), *generative structuralism* (Rieppel, 1990), *developmentalism* (Weber & Depew, 1996; Depew, 1998), *constructivism* (Oyama, 2000; Lewontin, 2000; Van de Vijver, Van Speybroeck & Vandevyvere, 2003) or *developmental system theory* (Griffiths & Gray, 1994; Van Speybroeck, 2000; Robert, Hall & Olson, 2001). The differences attributed to these various appellations are far from clear. In this dissertation, I will assume that the distinctions between these terms are irrelevant to the matters discussed here. I will refer to *structuralism*, which has well defined foundations (Piaget, 1972) or *generative structuralism* (in the sense of Rieppel, 1990, which was inspired by the rational morphologist school of pre-Darwinian times, whose famous figures were for instance Richard Owen, Etienne Geoffroy St Hilaire and Karl Von Baer, see chapter 1).

II. Toward a theory of forms

The *generative structuralist approach* of morphogenesis generally seeks to highlight the 'laws of form', that is how developmental systems determine the variation of organismal forms on short and long time scales. Surely, the term 'law' is too strong a statement. According to Van der Steen & Kamminga (1991, p.445-446), *"[a] statement is a law if it satisfies the following criteria: (i)- it is general in the sense that it contains a universal quantifier; (ii)- it is general in the sense that it does not mention particular individuals, times or places; (iii)- it has empirical content; (iv)- it is well-confirmed, and (v)- it is well entrenched (i.e. it belongs to a theory)"*. These authors go on to say that "*[t]he distinction between laws and natural history is not a very sharp one. If there are differences in this respect between physics and biology…, they will be matter of degree"*. In biology, however, we cannot expect much more than generalizations that may only be applied cautiously to a particular group of animals, at a particular time, thus contradicting criterion ii. One reason for it can be that the 'laws' themselves are expected to change during evolution ('the evolution of development', e.g. see Newman, 2002). The term 'law' has been much criticized because it seems to imply that development follows rules which are allegedly immutable, just like gravity. In order to prevent these misunderstandings, the word *rule* will be used instead.

Structuralism is opposed to atomizaion, which consists in attempting to reduce the explanation of phenomena to their lower hierarchical

levels of organization and necessarily implies a linear chain of causation between these levels. 'Generative structuralism' is close to organicism[1], in the ways described by Gilbert & Sarkar (2000, p. 2): *"Organicism claims ... that top-down and bottom-up approaches must both be used to explain phenomena… The properties of any level depend both on the properties of the parts "beneath" them and the properties of the whole into which they are assembled"*.

During the last decades, theoretical models of morphogenesis have allowed the identification of some *'construction rules'* from experimentally well-studied developmental systems (e.g. gastrulation, segmentation, neurulation, limb morphogenesis). In that way, several generalizations about how shapes may be generated have been suggested (see chapter 1 and its appendix). The non-straightforward relation linking the genotype with the phenotype has gained a better understanding from these studies. Moreover, mathematical modelling has been proved essential to inquire the role of development in affecting the direction of evolutionary change.

(1) Constraints

The most basic concept is that biological forms are *constrained* by the possibilities that the rules of chemistry, physics and geometry allow (Thompson, 1952). In other words, the set of theoretically possible forms is bounded by the way these forms can be generated.

Historically, the concept of *'developmental constraints'* has been used to challenge the 'Modern Synthesis' which denied that development could play any role in determining the direction of evolution. In particular, 'developmental constraints' were used to argue against the view that, without selection, phenotypic variation would be random (e.g. Alberch, 1980 and see chapters 1 & 2). But, it appears that 'random phenotypic variation' is not an easily definable concept (although it may seem at first sight). Moreover, different meanings have been given to 'random' in the synthesis (Eble, 1999). These complications put apart, it appeared that in practice, the 'Modern Synthesis' assumed that variation was gradual and 'in every direction', a view which has been much debated, at least since the late 70's under the umbrella of 'developmental constraints'.

Sometimes, the term 'constraints' is also used in a somehow different meaning than discussed above. In this usage, constraints are similar to the mathematical constraints that correspond to the initial and boundary conditions of the system under study. In mathematical models, the behaviour of developmental systems is described, characterized and predicted thanks to the rules of interaction between molecules, proteins, cells and / or tissues under a particular set of constraints (initial and boundary conditions). Without such constraints, the behaviour of a system cannot be predicted. In this view, constraints are given a generative role.

When critics of the 'Modern Synthesis' pointed out that not every kinds of variation were developmentally possible (first sense), they were

1 On the differences between organicism and structuralism, El-Hani & Emmeche (2000, p. 239-240, my emphasis) note that *"[i]n the present context it is important to note, first, the agreement between mainstream organicism and structuralism in understanding the organism as a real phenomenon and as an emergent structure with special dynamic properties...Second, one should also note a divergence concerning the* ***ontology of organic life****, to the effect that the neo-Darwinian Ernst Mayr as an organicist [*though only using the label organicism in his 1997 book*] emphasizes the existential and history-bounded* ***uniqueness*** *of living processes (such processes being dependent upon the historically evolved `genetic programme') whereas a structuralist like Goodwin tends to see the generic principles of pattern formation in development as emergent physical structures,* ***not necessarily peculiar*** *to living organisms as such but universally existing in Nature wherever the right boundary conditions are present."*

arguing that development was limiting the range of possible variation accessible to natural selection on one side, and that development was creative on the other side (second sense). In that way the first and second meanings of 'constraints' partially overlap, the first building extensively on the second.

Probably because of this ambiguity in the term 'constraints' (limiting / creative role), it seems more or less implicitly advocated that this term be avoided (Arthur, 2004). The now 'uncontroversial'[2] position that most 'evo-devoists' seem to endorse is that first development determines the directions and extent of variation, then, selection can choose among these possibilities (Salazar-Ciudad, 2006). It may be a trivial claim; yet, some authors persist in denying the role of constraints in evolutionary theories, thus allowing the maintenance of the adaptationist program (see chapter 2).

Some conflict between Neo-Darwinism and structuralism comes from the fact that the former is a *variational theory* of change where organisms are treated as passive objects whereas the latter is a *transformational theory* of change where organisms are viewed as the subjects of their own changes (Levins & Lewontin, 1985). According to the 'Modern Synthesis', organisms are viewed as the passive objects which are acted upon by genes and by natural selection, so they become irrelevant to evolutionary theories. As put by Goodwin (2000, p. 16), *"for what we seem to be faced with is a basic disjunction: neither historical narratives linked to genetic change, nor a detailed understanding of molecular composition, can explain the emergent morphological order observed at the level of organisms nor the logical (i.e. intrinsic) relationships between different types of organism that make taxonomy possible"*. Once one recognizes these fallacies, it becomes clear that we need a theory of form that relies on the non-linear dynamics of development to satisfactorily account for organisms' evolution (their generation, their invariant properties and their variation).

(2) Conceptual framework

The elaboration of an appropriate theoretical framework that integrates the non-linear dynamics of development as its fundamental basis is a challenge that defies all superlatives. This objective implies a drastic shift in the way causality is generally understood. Several authors are waiting for a 'revolution' in biology, one in which the existing genetic determinism will give way to a new understanding of the complexity of living organisms (Strohman, 1997).

Analogies between the behavior of biological and non-biological systems highlight some similarity in the rules governing the *organization* of living and inert matter. Two properties of developmental systems are robustness and capacity for change. Development is viewed as *generic*, that is, whatever the peculiar mechanism involved, developmental systems are expected to exhibit an intriguing combination of robustness and capacity for change. The interesting point is that these two antagonistic properties of developmental systems (robustness and variation) can be viewed as quasi-universal properties of *'dynamic systems'*. Complex systems are defined by the *interactions* between the systems components. These interactions generally exhibit non-linear dynamics (at some scale of observation). *Feedbacks* between levels of organization and *context-dependence* are two basic characteristics of developmental systems and more generally

2 Note that this postion implies a linear reasoning ("first" and "then") and that things are more complicated than one would think at first sight.

open systems (systems which exchange energy or matter with the 'outside' of the system).

The principle of organization stems from the interaction between 'parts' and 'whole' which are viewed as reciprocally constitutive (Webster & Goodwin 1982). *Emergence* is defined as the phenomenon by which a system of interactive 'parts' acquires new properties that cannot be understood as the simple superposition of the individual properties of these 'parts'. The concept of emergence naturally leads to consider that causation is *decentralized* across hierarchical levels of organization (because of feedbacks). A consequence is that higher levels of organization are *irreducible* to lower levels. This view challenges atomist theories of biological organization (such as the 'Modern Synthesis'). More and more evidence points to the conclusion that higher levels can causally affect the lower levels they structurally include as well as the reverse (upward and downward causation, see chapter 1). This circular causation is of course a conceptual, philosophical and technical challenge that goes far beyond the scope of this dissertation. Other difficulties lie in the fact that we currently have no reliable way to define and quantify robustness and capacity for change. Few studies have attempted to do that, and much work about it is to be done in the future.

(3) Morphogenetic models and model organisms

An essential aim (though not the only one) of theoretical models of morphogenesis is to mimic the basic features of the underlying morphogenetic mechanisms in order to gain insight on the *minimal hypotheses* that one has to assume to account for the *main outcomes*.

Many models of morphogenesis seem to have a common logical structure. They use a few sets of interaction rules that appear to be conserved in a variety of contexts. Consequently, many of the emergent properties of these models are generic, meaning that these properties are not specific to a particular mechanism. The *quasi-universality* of models of morphogenesis can be viewed as their main advantage and their Achilles heel at the same time. These models are used to understand the general dynamics of the system under study but the peculiar mechanisms involved can only be investigated via experimentation.

Experimental developmental biology has mainly focused on model organisms, such as the bacteria *Escherichia coli*, the nematode *Ceanorhabditis elegans*, the fruit fly *Drosophila melanogaster*, the sea urchin, the zebrafish, the frog *Xenopus laevis*, the chick, the mouse *Mus musculus* and the plant *Arabidopsis thaliana*. These model organisms have been chosen for various reasons: representation of major phyla, rapid developmental rate, short generation time, translucent body, easy manipulation, etc… In recent years, studies on development tended to extend to non-model organisms to allow a comparative approach of developmental genetics in closely related species as well as broad-scale comparisons between 'primitive' and 'derived' species. But the reliance of evo-devo on a relatively small number of model organisms has been viewed as a practical barrier between evolutionary biology and 'evo-devo' (Amundson, 2005). Typically, evolutionary studies have focused on a wider range of animals. For obvious reasons, paleontology extensively focuses on animals with high preservation potential and extensive diversity (e.g molluscs, vertebrates, conodonts, foraminifers, etc).

III. Molluscs

Molluscs are well studied by paleontologists and ecologists. The study of evolutionary changes occurring in mollusc lineages relies extensively on the interpretation of shell morphologies. Important taxonomic features of molluscs include the shape of the aperture, the degree of coiling of the shell tube, the ornamentation (ribs, tubercles, spines, keels) and growth features (growth halts, constrictions, varices). The evolution of the molluscan shell is characterized by frequent convergences in form and ornamentation. As a consequence, the recognition of transformation of one shape into another crucially depends on the knowledge of how these shell shapes are generated.

However, relatively few is known about molluscs development, if one 'excludes' early ontogeny (e.g. spiral cleavage, gastrulation, shell field formation, larval development, etc…see for instance Kniprath, 1978; Bandel & Boletzky, 1979; Kniprah, 1981; Bandel, 1986; Boletzky, 1989; Hopkins & Boletzky, 1994; Boletzky, 2003; Chirat & Boletzky, 2003) and processes of biomineralization (Simkiss, Wilbur & Wilbur, 1989; Marxen *et al.*, 1998; Sud *et al.*, 2001; Marxen, 2003; Zhang *et al.*, 2003; Lin & Meyers, 2005; Addadi *et al.*, 2006; Nudelman *et al.*, 2006).

In particular, the mechanisms underlying molluscan accretionary shell growth remain poorly understood. The secretion of the molluscan shell is achieved by the mantle, a soft sheet of connective tissue covered by an epithelium. During shell growth, the mantle, lying inside the shell, extends slightly beyond the aperture to add a shell increment to the margin (accretionary growth). Thus the growth, the shape and the direction of the shell increments added during each growth episode are almost equivalent to the state of the mantle edge at the same time.

Probably because of their aesthetic beauty, molluscan shells have fascinated humans for centuries (Fig. 1). But more importantly, their simple shape has been the subject of many theoretical works, emphasizing the regularity of accretionary growth which often conforms well to logarithmic spiral coiling (see chapter 3). Moreover, the different kinds of models of morphogenesis proposed so far have been applied to molluscan shell morphogenesis (see introduction in part II of this dissertation). The preservation of the ontogeny of the shell due to its accretionary growth is an undeniable advantage of molluscs to study the relationship between evolution and development.

The comparison between different clades of molluscs can be informative with regards to the basic rules of accretionary growth. In particular, it has been pointed out that common rules of accretionary growth could underlie the morphogenesis of the shell and its evolution in ammonoids and gastropods (Bucher, 1997; Checa, Jimenez-Jimenez & Rivas, 1998). Evidences come from the comparison of intraspecific patterns of covariation between shell characters, from the description of changes occurring at maturity and from the analysis of teratological shells with malformations caused by injuries or change in living conditions.

(1) Teratology

Teratological shells may provide a useful source of information about the way development generally proceeds. For instance, planispiral ammonites that were infested by epizoans during their life time exhibit alterations of their *coiling geometry* (Checa, Okamoto & Keupp, 2002).

Fig. 1: Snail drawings on the walls of Drepung monastery, Lhassa, Tibet. Photographs by Nicolas Goudemand.

These authors point out that, most commonly, the epizoans settled on the venter of ammonoids and constituted an obstacle to the subsequent growth. This disturbance probably initiated changes in the hydrostatic conditions of the ammonite and caused a lateral shifting of the growth direction compared to the previous whorl in attempts to avoid the obstacle. Using a hydrostatic model, these authors show that the shell tube should periodically cross the venter, thus leading to zig-zag coiling, if the ammonite tried to maintain the growth direction perpendicular to the substrate. If the epizoan was positioned on the midventer, the whorl could be detached from the previous whorl. Under constant growth direction relative to the substrate, a lateral placement of the epizoan would rather result in trochospiral coiling, especially if the epizoan had a certain non-negligible weight, which could cause the tilting of the ammonite.

A similar role for life orientation in determining the growth direction has been experimentally tested in gastropods. In the benthonic freshwater Planorbidae (Gastropoda), specimens experimentally altered by extra weights on one side of the shell revealed that the growth direction remained perpendicular to the substrate (Checa & Jimenez-Jimenez, 1997). Similarly, the benthic prosobranch gastropods exhibiting a tangential aperture with regards to the coiling axis have been shown to live with the aperture parallel to the substrate (Linsley, 1977). These gastropods have the ability to regulate the amount of torsion/detorsion of the foot to place the centre of gravity of the shell and body over the midline of the cephalopodial mass, thus allowing the

maintenance of a constant life orientation. A well known example of the influence of change of mode of life on shell morphology is provided by the gastropod *Distorsio*, which, once settled on the substrate display distorted coiling.

In ammonoids, regenerated shells after damage are often found (Guex, 1967; Guex, 1968; Bayer, 1970; Landman & Waage, 1986; Bond & Saunders, 1989; Hammer & Bucher, 2005b). Particularly, some changes in the *ornamental features* have been described in response to the location of injuries reaching the mantle (Guex, 1967; Guex, 1968; Bayer, 1970; Hammer & Bucher, 2005b). For example, some shells with a ventral keel associated with ribs on the flanks can loose their keel in response to a wound located on the venter. Then, the ribs in the post-damaged shell cross the venter whereas they were before interrupted by the keel. Some other shells bearing bifurcating ribs on the venter rather construct simple ribs after being damaged on one side. These examples are described in terms of *"ornamental compensation"* (Guex, 1967; Guex, 1968). This phenomenon, like Buckman's law of covariation (see below), can be seen as a generic outcome of modes of shell growth, whether one interprets such results in terms of reaction-diffusion models (Guex *et al.*, 2003; Hammer & Bucher, 2005b), or in terms of mechanical effects (Hammer & Bucher, 2005a).

In Muricidae, ornamental features may also be greatly modified in some specimens sometimes as a consequence of damages of the mantle (Fig. 2; see also Houart, 2001). In *Bolinus brandaris* Linné 1758, the teratological specimens are particularly remarkable with respect to the extremely limited extent of the intraspecific variation. In this species, the number of rows of spines, as well as the number of spines per whorl is highly stable. But there exists a small proportion of specimens with a higher number of rows of spines[3]. When supernumerary rows of spines are present, spines may look typical or may be larger, recurved, subdivided and/or more opened (Fig. 2, specimens 1 and 2). For instance, specimen 1 exhibits three rows of spines on the body whorl and the second one from the umbilical line is subdivided. Specimen 2 exhibits four rows of spines on the body whorl and the second one from the umbilical line is subdivided too. Note that the subdivision of these spines mimics the spine morphology of related species ('foliated spines'). The presence of injuries in these two specimens cannot be ascertained, since no marks of injuries are visible on the last whorl. But in some specimens, the addition and subdivision of rows of spines in response to injuries is clear (Fig. 2, specimen 3). For instance, after a large breakage of the aperture and the siphon (black arrow), specimen 3 exhibits a subdivision of the first row of spines from the umbilical line and the spatial periodicity of growth halts is lost. In this specimen, the region of the mantle which was related to the normal second row of spines from the umbilical line slightly shifted to the posterior part of the aperture (white arrow) in the three shell increments immediately after damage.

3 Among about 100 kg of *B. brandaris* fished during a visit in Banyuls-sur-Mer, I found 2 variants with three spines and 1 with four spines. The spines looked typical and no scar has been observed.

Fig. 2: Teratological specimens of *Bolinus brandaris* from Malaga (Spain), trawled by fishermen at 30-40 meters depth (1-4). **1**: a variant with three rows of spines on the body whorl. **2**: a variant with four rows of spines on the body whorl. The presence of a precocious wound in **1** & **2** is not ascertained. Arrows: subdivided spines. **3**: shell anomalies in response to a breakage of the aperture and siphon (black arrow, contour breakage in white). Note the two step displacement of the mantle after the breakage and the closely spaced growth halts (white arrow heads). **4**: shell anomalies in response to a breakage of the aperture (arrow, breakage in white). Note the change in whorl overlap and approximated growth halts. **5**: shell repair in response to a damage not affecting the aperture (breakage in white). Note that a growth halt is built just next the previous one. Inderterminated species. Photographs by Rosi Roth (PIMUZ).


1
2
4
5
3
3cm

In the subsequent growth increments, this row of spines tends to move more anteriorly probably as a consequence of the regrowing of the siphon. This illustrates that the mantle withdrew into the shell just after the damage and became progressively stretched during the following growth steps. Shell damages can also cause the spines to be lost. For instance, after a breakage of the aperture (Fig. 2, arrow), specimen 4 is nearly completely smooth. This change is accompanied by a slight increase in whorl overlap and the losing of the spatial periodicity of growth halts, indicating that the wound reached the mantle and probably affected its visco-elastic properties. A moderate modification of the shell in response to a shell breakage away from the aperture is illustrated in specimen 5 (Fig. 2). Note that the spiral strigations of the repaired shell are not strictly concordant with the remains of the shells built before damage. A growth halt is built just next the remains of the growth halt built before damage. The next increment looks normal. This points out that the mantle edge has not been (seriously) damaged and that the repaired shell has not been secreted by the mantle edge.

(2) Patterns of covariation

(a) Buckman's laws of covariation

In ammonoids, extensive intraspecific variation has been described by numerous authors (e.g. Silberling, 1956; Westermann, 1966; Kennedy & Cobban, 1976; Dagys & Weitschat, 1993; Dagys, Bucher & Weitschat, 1999). Whithin species, correlations between the intensity of ornamentation, the lateral compression of the aperture and the degree of whorl overlap have been noticed in Jurassic ammonites (Westermann, 1966), Triassic boreal ammonoids (Rieber, 1972; Dagys & Weitschat, 1993, Checa *et al.*, 1996; Bucher, 1997; Dagys, Bucher & Weitschat, 1999; Monnet & Bucher, 2005; Hammer & Bucher, 2005a) and Cretaceous ammonites (Kennedy & Cobban, 1976). These correlations are known as the *'Buckman's laws of covariation'* (Westermann, 1966). Figure 3 illustrates these covariations in an assemblage of juveniles of *Amaltheus margaritatus* from Jurassic. In this assemblage, one can see that the most robust variants (a_1, a_2, b_1, b_2, c_1, c_2) display relatively few strong ribs, a small whorl overlap and widely spaced septa, whereas compressed variants (a_3, a_4, b_3, c_3) exhibit numerous faint ribs, a high whorl overlap and closely spaced septa. During ontogenesis, specimens tend to display relatively narrower apertures, increased coiling and fainted ornamentation, so that the most extreme variation is observed in the juveniles samples.

It seems that the negative correlation between the compression of the aperture and the intensity of ornamentation can be satisfactorily accounted for by assuming that lateral rib heights increase isometrically with aperture width whereas ventral rib heights increase isometrically with aperture height (Hammer & Bucher 2005a, Fig. 4). Simple scaling relationships lead to produce proportionally stronger lateral ribs on depressed specimens than on compressed specimens which only exhibit strong ribs on venter (Fig. 4). Similar arguments should hold for the negative correlation between involution and intensity of ornamentation (Hammer & Bucher, 2005a). However, this simple model does not account for the negative correlation between the amplitude and spatial frequency of ribs, since compressed specimens often tend to have more closely spaced faint ribs (compare Fig. 3 and Fig. 4). Also, the negative correlation between whorl compression and septal spacing has not been accounted for from a morphogenetic point

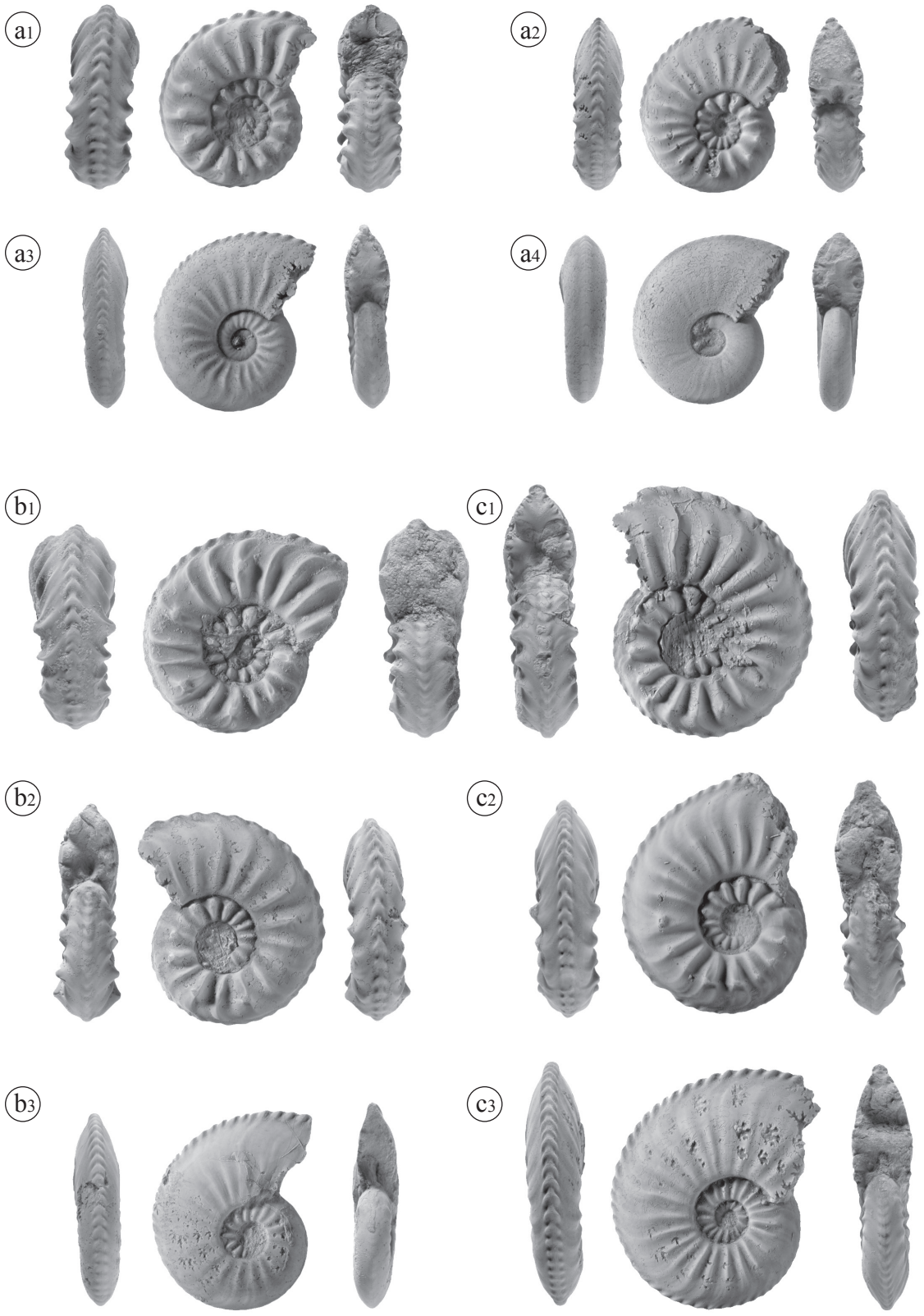


Fig. 3: Variation in an assemblage of juveniles of *Amaltheus margaritatus* from Jurassic. The specimens are ordered from the more robust to the more compressed for similar diameters in **a** to **c**. Note the specimens with the broadest apertures have few strong ribs, largely spaced septa and relatively small whorl overlap. To the contrary, the specimens with the tightest apertures exhibit numerous closely spaced faint ribs, approximated septa and high whorl overlap. On these internal moulds, growth halts are not visible. Photographs by Noël Podevigne (UCBL, Lyon).

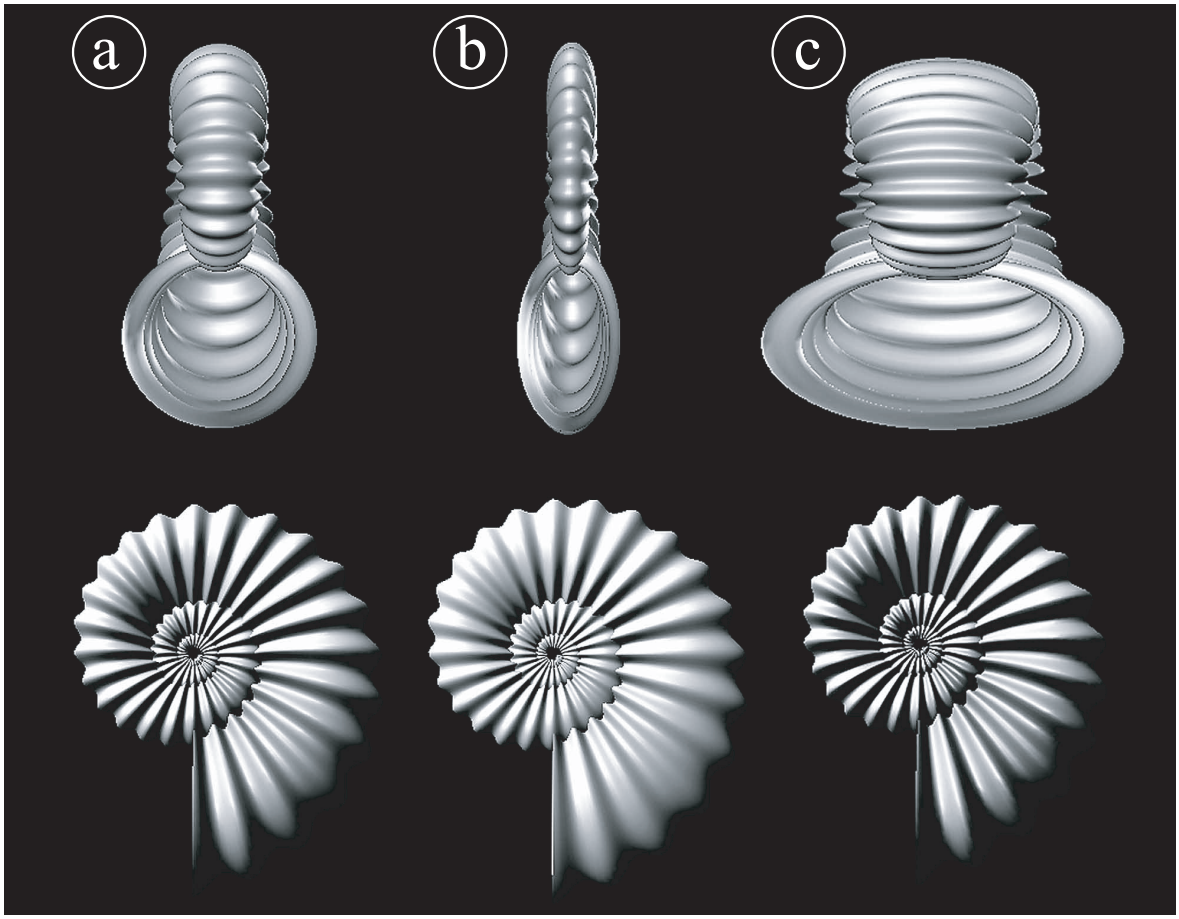


Fig. 4: Synthetic computer models of hypothetical ammonoids shells, illustrating Buckman's law of covariation as a simple case of proportionality. **Top**: apertural views. **Bottom**: lateral views. **a**: a shell with a circular aperture and equal amplitudes of lateral and ventral ribbing. **b**: same shell as in **a**, but laterally compressed by simple scaling of the lateral axis. The lateral ribs get proportionally weaker. **c**: same shell as in **a**, but laterally expanded (depressed). The lateral ribs get proportionally stronger. Note that ventral rib amplitude stays constant under lateral scaling, causing a variation in the ratio between ventral and lateral rib amplitude. From Hammer & Bucher (2005a).

of view. For instance, Hammer & Bucher (2006) loosely referred to heterochrony (the robust variants of *Amaltheus margaritatus* being regarded as paedomorphic compared to the compressed variants) and suggested a functional explanation in terms of hydrostatics properties to account for the more closely spaced growth halts in compressed variants.

(b) Growth halts and allometry

Other patterns of variation of shell shape and its associated growth features, particularly *growth halts*, in ammonoids and gastropods also point out that similar rules of accretionary growth underlie the morphogenesis of the shell and its evolution in both clades (Bucher, 1997). At maturity, the spacing between successive growth halts tends to decrease in ammonoids and gastropods. Growth halts approximation is generally accompanied by a change in aperture shape and/or coiling. For instance, in the gastropod *Epitonium scalare*, the shell is isometric (or nearly so) until maturity which is recorded in the shell by a more elliptic aperture and a few (about 5) approximated growth halts. In Muricidae, growth halts are also more closely spaced at maturity. For instance, in *Bolinus brandaris*, a decrease in the length of spines is coinciding with two approximated growth halts (personal observation). Bucher (1997) notes also that in *Murex haustellum*, the

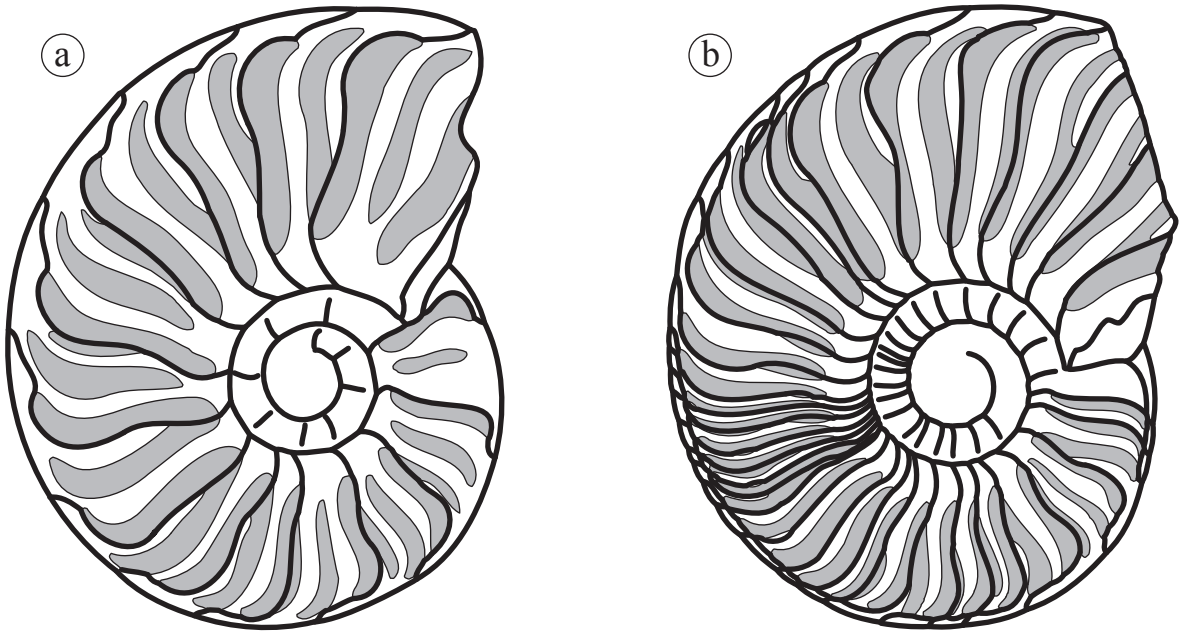


Fig. 5: Lateral view of two variants of *Gymnotoceras rotelliformis*: **a**: depressed variant. **b**: compressed variant. The shaded areas represent ribs, the dark lines, growth halts. Growth halts, ribs and septa (not shown) are all more closely spaced in the more compressed specimen (**b**). Redrawn from Bucher (1997).

first growth halt, at about 3.5 whorls after the embryonic constriction, is coinciding with an apertural shape change. Closely spaced growth halts at maturity are also quite frequent in ammonoids, for instance in *Parafrechites* and in *Gymnotoceras*.

More generally, Bucher (1997) suggests that a regular/irregular spacing between growth halts ('constant' angle or not) during ontogeny could be related to isometric/allometric growth of the aperture. Throughout ontogeny and within populations, aperture shape and ornamentation tend to covary with the spacing between growth halts (Bucher, 1997). For instance, in *Gymnotoceras rotelliformis*, growth halts and ribs are more closely spaced in the compressed variants than in the depressed variants (Fig. 5, see also Fig. 3). Specimens tend to become more compressed during ontogeny while the number of growth halts per whorl tends to increase. Also, during ontogeny, the spacing between growth halts is reflected in the spacing between septa (Bucher, 1997; see also Bucher & Guex, 1990; Bucher *et al.*, 1996 for documentation in *Parafrechites* and *Eotetragonites*). These patterns of variation between growth halts, ornamentation, aperture shape and septa are quite frequent in the Triassic subfamilies Berichitinidae and Paraceratitinae (Monnet & Bucher, 2005).

One goal of this dissertation is to explore whether a similar pattern of covariation between aperture shape, intensity of ornamentation and spacing between growth halts is to be found in gastropods as well (chapter 5). One interest is to find out if documentation of modes of growth in gastropods (e.g. frequency and amplitude of pulses of growth) could support the view according to which some recurrent patterns of covariation in ammonoids species are the result of rules of growth tied to basic constraints of accretionary growth common to both clades. Another interest is the relationship between shape, growth rates and age, a point that is difficult (if not impossible) to study on ammonoids.

(c) Growth rates and shape

Extensive phenotypic variation is well known in living intertidal gastropods, especially in the Littorinidae (e.g. Vermeij, 1980; Kemp & Bertness, 1984; Boulding & Hay, 1993; Chapman, 1995; Johannesson, Rolán-Alvarez, Erlandsson, 1997; Yeap, Black & Johnson, 2001; Carballo, Caballero & Rolan-Alvarez, 2005). Various transplant and growth experiments in field and laboratory pointed out that natural selection could not be the only factor responsible for the reported correlations between some shell traits and some environmental aspects. *Phenotypic plasticity*, defined as the capacity of organisms to alter their morphological and life-history traits (e.g. growth rates) in response to their living conditions, slowly emerged as a concept which could account for much of the patterns of variation among mollusc ecomorphs, populations and species (e.g. Palmer, 1992). Interestingly, several studies suggested a relationship between growth rates and shell shape (e.g. Vermeij, 1980; Kemp & Bertness, 1984; Boulding & Hay, 1993; see chapter 4).

IV. Goals of this dissertation

The issues addressed in this dissertation concern the description of the relationships between growth and form. How do growth dynamics link to allometric relationships and covariation between characters? This dissertation was originally motivated by the question of the origins of the important structuration of shell variation in some ammonoid species, subsumed as the 'Buckman's laws of covariation'. In particular, a goal was to explore whether similar patterns of covariation would exist in gastropods, especially covariations between growth halts spacing, aperture shape and intensity of ornamentation.

Obviously, the species studied naturally had to exhibit growth halts and ornamental features (spines). Muricidae were ideal candidates for this purpose. It turned out that an egg mass of *Hexaplex (Trunculariopsis) trunculus* (Linnaeus, 1758) has been kindly provided by the Observatoire Océanologique de Banyuls-sur-mer (France). These newly hatched juveniles have been used to gain knowledge on the timing of growth halts formation (growth rhythm, pulses of growth), to analyse individual ontogenetic trajectories, to describe patterns of covariation, to quantify the amount of intraspecific variation and to test if any correlations between shape and growth rates could exist (chapter 5). These issues have also been investigated theoretically. A model, based on a modified version of that proposed by Hammer & Bucher (2005b), was used to understand how hypothetical growth rules were reflected in the generated patterns of ontogenetic allometry (chapter 3) and patterns of variation among populations (chapter 4). The insights provided by this theoretical approach greatly helped the interpretation in the empirical study (chapter 5).

This dissertation represents an attempt to shed light on some aspects of the following questions:

- How can generic models inform us about the generation and evolution of structures of particular size and shape and *vice-versa*?

- What kind of morphological variation is expected given some basic rules of growth?

- What kind of rules could underlie the observed patterns of variation?

V. Thesis outline

I have chosen to divide this thesis in two parts: the first one serves as a general introduction to the problems and prospects of evolution and development, whereas the second deals exclusively with growth and variation of molluscan shell shape. Obviously, these parts are reciprocally constitutive. Questions of the second part motivated the writing of the first one, which inferred the second one in return.

Part I: Evolution and development: problems and prospects

Chapter 1 provides an overlook of 'Evo-Devo': its historical relationships with the 19th century rationalists, its decline following the establishment of the 'Modern Synthesis', its rebirth thanks to the structuralist critics of the 70's-80's and its past and present connections with genetics, with Neo-Darwinism and with computational models. This chapter critically discusses diverse models of morphogenesis, namely the 'positional information' concept (Wolpert 1969), the reaction-diffusion models (Turing, 1952) and the mechanical models (His, 1888). 'Generative structuralism' is reassessed as a suitable framework to resolve some problematic issues regarding the conceptualization of development and evolution. It is believed that this approach can allow one to appreciate the relative amount of generality and contingency in developmental systems, a problem that plagued the history of 'evolutionary theory' under the functionalist/ structuralist debates.

The following appendix is a book review that recalls some basic principles of biological organization and theoretical modelling. In particular, the contribution of discrete models of morphogenesis (known as cellular automata) is critically reviewed and the benefits of these models are outlined.

In **chapter 2**, it is discussed how the taking into account of the 'complexity of gastropods shell coiling' challenges the atomistic conception of biological organization. In this chapter, the assumptions underlying the 'Neo-Darwininan paradigm' and the outdated metaphors referring to genetic or development programs are discussed. This chapter can be viewed as an example of how current knowledge on development, especially the fact that morphogenesis is endowed with generative rules, can inform evolutionary theories. Thus, this chapter goes beyond the level of intra-specific variation, which is the focus of the next part of this dissertation.

Part II: Molluscan shell shape: growth models and patterns of variation

Chapters 3 and 4 propose and discuss a null model of molluscan shell growth, relying on modifications of the model proposed by Hammer & Bucher (2005b).

In **chapter 3**, the model assumptions are exposed and compared to other shell models proposed so far. The approach undertaken here departs from earlier proposed models of shell growth, in the sense that the starting point of these models generally was the logarithmic spiral model. Here, exactly the contrary holds. Simple rules related to the scaling and relative arrangement of successive growth increments are postulated first. Then, the conditions satisfying the simple logarithmic spiral model are derived. This model can be viewed as a generalization of earlier proposed models of shell growth. It illustrates some fundamental geometrical properties of logarithmic spirals, in particular

the close relationship between the size and the shape of growth increments (discrete model). More generally, this model highlights the role of growth rates and timing in the generation of allometries.

Chapter 4 builds upon the model discussed in chapter 3. This model is used to discuss variation at the population level. This model is viewed as a convenient mean to generate non-linearities, while retaining the link between the derived patterns of intra-population variation and the variability in model parameters. As far as possible, the hypotheses of this model and its predictions are confronted to the empirical observations and data from the literature. Additionally, randomization of time of measurements simulates populations composed of variably aged specimens, thus generating empirical-like growth data. It allows one to investigate how mixing of different 'age classes' can impinge on the variation observed in a population. The model also allows the comparison between static and ontogenetic allometry.

Chapter 5, which is at the end of this dissertation but which is nevertheless at its core, investigates more deeply the ontogenetic patterns of covariation between shells characters of a population of gastropods (*Hexaplex trunculus*, Muricidae) originated from a single egg mass and reared in laboratory for about one year and a half. This study can be called *longitudinal*, since it presents individual growth curves and ontogenetic trajectories. The time spent on a growth halt seems to increase exponentially with age. Variation in growth rhythm (frequency and amplitude of pulses of growth), in growth rates (e.g. mm shell length per day) and in shape of growth curves (detectable quiescent phases or not) is extensive. This study highlights a covariation between growth rhythm, growth halts spacing, aperture allometry and intensity of ornamentation. In particular, variation in growth rhythm is regarded as critical in generating the observed covariation between growth halts spacing and ornamentation. The growth vector model, discussed in chapters 3 & 4, is used to simulate the formation of growth halts phenomenologically. Regardless of variation in growth rates and variation in growth rhythm during ontogeny, this model is able to account for the empirically observed covariations between shell characters. These covariations are proposed to mainly result from simple scaling between the aperture dimensions and the lengths of shell segments between successive growth halts.

The theoretical and experimental framework developed here could assist in formulating and testing new hypotheses of growth of molluscan shells. It paves the way toward the development of data-driven mathematical models which could greatly help in interpreting empirical data. In particular, it would be possible in the future to integrate growth curves (just like those illustrated in chapter 5) into the growth vector model. Thus, this model could facilitate the comparison of theoretical and empirical data in the future, and perhaps help interpreting them in a developmental, ecological and evolutionary context.

VI. References

Addadi, L., Joester, D., Nudelman, F. & Weiner, S. (2006). Mollusk shell formation: a source of new concepts for understanding biomineralization processes. *Chemistry - a European Journal* **12,** 981-987.

Alberch, P. (1980). Ontogenesis and morphological diversification. *American Zoologist* **20,** 653-667.

Amundson, R. (2000). Embryology and evolution 1920-1960: worlds apart? *History and Philosophy of the Life Sciences* **22,** 335-352.

Amundson, R. (2005). *The Changing Role of the Embryo in Evolutionary Thought: Roots of Evo-Devo*. Cambridge University Press Cambridge.

Arthur, W. (2004). The effect of development on the direction of evolution: toward a twenty-first century consensus. *Evolution & Development* **6,** 282-288.

Bandel, K. (1986). The ammonitella: a model of formation with the aid of the embryonic shell of archaeogastropods. *Lethaia* **19,** 171-180.

Bandel, K. & Boletzky, S. V. (1979). Comparative study of the structure, development and morphological relationships of chambered cephalopod shells. *Veliger* **21,** 313-354.

Bayer, U. (1970). Anomalien bei Ammoniten des Aaleniums und Bajociums und ihre Beziehung zur Lebensweise. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **135,** 19-41.

Boletzky, S. V. (1989). Early ontogeny and evolution: the cephalopod model viewed from the point of developmental morphology. *Geobios***,** 67-78.

Boletzky, S. V. (2003). Biology of early life stages in cephalopod molluscs. In *Advances in Marine Biology, Vol 44*, vol. 44. *Advances in Marine Biology*, pp. 143-203.

Bond, P. N. & Saunders, W. B. (1989). Sublethal injury and shell repair in upper Mississippian ammonoids. *Paleobiology* **15,** 414-428.

Boulding, E. G. & Hay, T. K. (1993). Quantitative genetics of shell form of an intertidal snail: constraints on short-term response to selection. *Evolution* **47,** 576-592.

Bucher, H. (1997). Caractères périodiques et modes de croissance des ammonites: comparaison avec les gastéropodes. *Geobios* **20,** 85-99.

Bucher, H. & Guex, J. (1990). Rythmes de croissance chez les ammonites triasiques. *Bulletin des Laboratoires de Géologie, Minéralogie, Géophysique et du Musée géologique de l'Université de Lausanne* **308,** 191-209.

Bucher, H., Landman, N. H., Klofak, S. M. & Guex, J. (1996). Mode and rate of growth in ammonoids. In *Ammonoid paleobiology* (ed. N. H. Landman), pp. 408-461. Plenum Press, New-York.

Carballo, M., Caballero, A. & Rolán-Alvarez, E. (2005). Habitat-dependent ecotype micro-distribution at the mid-shore in natural populations of *Littorina saxatilis*. *Hydrobiologia* **548,** 307-311.

Chapman, M. G. (1995). Spatial patterns of shell shape of three species of co-existing littorinid snails in New South Wales, Australia. *Journal of Molluscan Studies* **61,** 141-162.

Checa, A., Company, M., Sandoval, J. & Weitschat, W. (1996). Covariation of morphological characters in the Triassic ammonoid *Czekanowskites rieberi*. *Lethaia* **29,** 225-235.

Checa, A. G. & Jimenez-Jimenez, A. P. (1997). Regulation of spiral growth in planorbid gastropods. *Lethaia* **30,** 257-269.

Checa, A. G., Jimenez-Jimenez, A. P. & Rivas, P. (1998). Regulation of spiral coiling in the terrestrial gastropod *Sphincterochila*: an experimental test of the road-holding model. *Journal of Morphology* **235,** 249-257.

Checa, A. G., Okamoto, T. & Keupp, H. (2002). Abnormalities as natural experiments: a morphogenetic model for coiling regulation in planispiral ammonites. *Paleobiology* **28,** 127-138.

Chirat, R. & Boletzky, S. V. (2003). Morphogenetic significance of the conchal furrow in nautiloids:evidence from early embryonic shell development of Jurassic Nautilida. *Lethaia* **36,** 161-170.

Dagys, A., Bucher, H. & Weitschat, W. (1999). Intraspecific variation of *Parasibirites kolymensis* Bychkov (Ammonoidea) from the Lower Triassic (Spathian) of Arctic Asia Mitteilungen *Geologisches - Paläontologisches Institut Universität Hamburg* **83,** 163-178.

Dagys, A. S. & Weitschat, W. (1993). Extensive intraspecific variation in a Triassic ammonoid from Siberia. *Lethaia* **26,** 113-121.

Depew, D. J. (1998). Darwinism and developmentalism: prospects for convergence. In *Evolutionary Systems: Biological and Epistemological Perspectives on Selection and Self-Organization* (ed. G. Van de Vijver, S. N. Salthe and M. Delpos), pp. 21-32. Kluwer Academic Publishers, Dordrecht.

Eble, G. J. (1999). On the dual nature of chance in evolutionary biology and paleobiology. *Paleobiology* **25,** 75-87.

El-Hani, C. N. & Emmeche, C. (2000). On some theoretical grounds for an organism-centered biology: Property emergence, supervenience, and downward causation. *Theory in Biosciences* **119,** 234-275.

Gilbert, S. F. & Sarkar, S. (2000). Embracing complexity:

organicism for the 21st century. *Developmental Dynamics* **219,** 1-9.

Goodwin, B. C. (2000). The life of form. Emergent patterns of morphological transformation. *Comptes Rendus de l'Académie des Sciences Série III-Sciences de la Vie-Life Sciences* **323,** 15-21.

Griffiths, P. E. (1996). Darwinism, process structuralism, and natural kinds. *Philosophy of Science* **63,** S1-S9.

Griffiths, P. E. & Gray, R. D. (1994). Developmental systems and evolutionary explanation. *Journal of Philosophy* **91,** 277-304.

Guex, J. (1967). Contribution à l'étude des blessures chez les ammonites. *Bulletin géologique de Lausanne* **165,** 1-23.

Guex, J. (1968). Sur deux conséquences particulières des traumatismes du manteau des ammonites. *Bulletin de la Societé vaudoise des sciences naturelles*.

Guex, J., Koch, A., O'Dogherty, L. & Bucher, H. (2003). A morphogenetic explanation of Buckman's law of covariation. *Bulletin de la Société Géologique de France* **174,** 603-606.

Hammer, O. & Bucher, H. (2005a). Buckman's first law of covariation: a case of proportionality. *Lethaia* **38,** 67-72.

Hammer, O. & Bucher, H. (2005b). Models for the morphogenesis of the molluscan shell. *Lethaia* **38,** 111-122.

Hammer, O. & Bucher, H. (2006). Generalized ammonoids hydrostatics modelling, with application to *Intornites* and intraspecific variation in *Amaltheus Paleontological Research* **10,** 91-96.

His, W. (1888). On the principles of animal morphology. *Royal Society of Edinburgh Proceedings* **15,** 287-298.

Ho, M. W. & Saunders, P. T. (1984). Pluralism and Convergence in Evolutionary Theory. In *Beyond Neo-Darwinism: An introduction to the new evolutionary paradigm* (ed. M. W. Ho and P. T. Saunders), pp. 3-12. Academic Press, London.

Hopkins, B. & Boletzky, S. V. (1994). The fine morphology of the shell sac in the squid genus *Loligo* (Mollusca, Cephalopoda): features of a modified conchiferan program. *Veliger* **37,** 344-357.

Horder, T. J. (2001). The organizer concept and modern embryology: Anglo-American perspectives. *International Journal of Developmental Biology* **45,** 97-132.

Houart, G. (2001). *A review of the recent mediterranean and northeastern atlantic species of Muricidae*. Evolver SRL.

Jenner, R. A. (2006). Unburdening evo-devo: ancestral attractions, model organisms, and basal baloney. *Development Genes and Evolution* **216,** 385-394.

Johannesson, K., Rolán-Alvarez, E. & Erlandsson, J. (1997). Growth rate differences between upper and lower shore ecotypes of the marine snail *Littorina saxatilis* (Olivi) (Gastropoda). *Biological Journal of the Linnean Society* **61,** 267-279.

Kemp, P. & Bertness, M. D. (1984). Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea*. *Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences* **81,** 811-813.

Kennedy, W. J. & Cobban, W. A. (1976). Aspects of ammonite biology, biogeography, and biostratigraphy. *Special Papers in Palaeontology* **17,** 1-94.

Kniprath, E. (1978). Growth of shell field in *Mytilus* (Bivalvia). *Zoologica Scripta* **7,** 119-120.

Kniprath, E. (1981). Ontogeny of the molluscan shell field: a review. *Zoologica Scripta* **10,** 61-79.

Landman, N. H. & Waage, K. M. (1986). Shell abnormalities in scaphitid ammonites. *Lethaia* **19,** 211-224.

Levins, R. & Lewontin, R. C. (1985). *The Dialectical Biologist*. Harvard University Press, Cambridge, MA & London

Lewontin, R. C. (2000). *The triple helix: gene, organism, and environment*. Harvard University Press, Cambridge, MA.

Lin, A. & Meyers, M. A. (2005). Growth and structure in abalone shell. *Materials Science and Engineering a-Structural Materials Properties Microstructure and Processing* **390,** 27-41.

Linsley, R. M. (1977). Some "laws" of gastropod shell form. *Paleobiology* **3,** 196-206.

Marxen, J. C., Becker, W., Finke, D., Hasse, B. & Epple, M. (2003). Early mineralization in *Biomphalaria glabrata*: microscopic and structural results. *Journal of Molluscan Studies* **69,** 113-121.

Marxen, J. C., Hammer, M., Gehrke, T. & Becker, W. (1998). Carbohydrates of the organic shell matrix and the shell-forming tissue of the snail *Biomphalaria glabrata* (Say). *Biological Bulletin* **194,** 231-240.

Mayr, E. (1963). *Animal species and evolution* Oxford University Press, Cambridge.

Monnet, C. & Bucher, H. (2005). New middle and late Anisian (Middle Triassic) ammonoid faunas from northwestern Nevada (USA): taxonomy and biochronology. *Fossils and Strata* **52,** 1-60.

Needham, J. (1930). *The sceptical biologist*. Norton, New York.

Newman, S. A. (2002). Developmental mechanisms: putting genes in their place. *Journal of Biosciences* **27,** 97-104.

Nijhout, H. F. & Emlen, D. J. (1998). Competition among body parts in the development and evolution of insect morphology. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 3685-3689.

Nijhout, H. F. & Wheeler, D. E. (1996). Growth models of complex allometries in holometabolous insects. *American Naturalist* **148,** 40-56.

Nudelman, F., Gotliv, B. A., Addadi, L. & Weiner, S. (2006). Mollusk shell formation: mapping the distribution of organic matrix components underlying a single aragonitic tablet in nacre. *Journal of Structural Biology* **153,** 176-187.

Oyama, S. (2000). *The Ontogeny of Information: Developmental Systems and Evolution*, Second Edition edition. Duke University Press, Duke.

Palmer, A. R. (1992). Calcification in marine mollusks : how costly is it? *Proceedings of the National Academy of Sciences of the United States of America* **89,** 1379-1382.

Piaget, J. (1972). *Le structuralisme*, Fifth edition. Presses universitaires de France, Paris.

Resnik, D. (1994). The rebirth of rational morphology: process structuralisms philosophy of biology. *Acta Biotheoretica* **42,** 1-14.

Rieber, H. (1972). Die Triasfauna der Tessiner Kalkalpen. XXII. Cephalopoden aus der Grenzbitumenzone (Mittelere Trias) des Monte San Giorgio (Kanton Tessin, Schweiz). *Schweizerische Paläontologische Abhandlungen* **93,** 1-95.

Rieppel, O. (1990). Structuralism, functionalism, and the four Aristotelian causes. *Journal of the History of Biology* **23,** 291-320.

Robert, J. S., Hall, B. K. & Olson, W. M. (2001). Bridging the gap between developmental systems theory and evolutionary developmental biology. *BioEssays* **23,** 954-962.

Salazar-Ciudad, I. (2006). Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development? *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution* **306B,** 107-125.

Silberling, N. J. (1956). *Trachyceras* zone in the Upper Triassic of the western United States. *Journal of Paleontology* **30,** 1147-1153.

Simkiss, K., Wilbur, K. M. & Wilbur, K. M. (1989). *Biomineralization cell biology and mineral deposition*. Academic Press, San Diego [etc.].

Smith, K. C. (1992). Neo-Rationalism *versus* Neo-Darwinism: integrating development and evolution. *Biology & Philosophy* **7,** 431-451.

Soto, A. M. & Sonnenschein, C. (2005). Emergentism as a default: cancer as a problem of tissue organization. *Journal of Biosciences* **30,** 103-118.

Stern, D. L. & Emlen, D. J. (1999). The developmental basis for allometry in insects. *Development* **126,** 1091-1101.

Strohman, R. C. (1997). The coming Kuhnian revolution in biology. *Nature Biotechnology* **15,** 194-200.

Sud, D., Doumenc, D., Lopez, E. & Milet, C. (2001). Role of water-soluble matrix fraction, extracted from the nacre of *Pinctada maxima*, in the regulation of cell activity in abalone mantle cell culture (*Haliotis tuberculata*). *Tissue & Cell* **33,** 154-160.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **237,** 37-72.

Van de Vijver, G., Van Speybroeck, L. & Vandevyvere, W. (2003). Reflecting on complexity of biological systems: Kant and beyond? *Acta Biotheoretica* **51,** 101-140.

Van Der Meulen, M. C. H. & Carter, D. R. (1995). Developmental mechanics determine long bone allometry. *Journal of Theoretical Biology* **172,** 323-327.

Van der Steen, W. J. & Kamminga, H. (1991). Laws and natural history in biology. *British Journal for the Philosophy of Science* **42,** 445-467.

Van Speybroeck, L. (2000). The organism: a crucial genomic context in molecular epigenetics? *Theory in Biosciences* **119,** 187-208.

Vermeij, G. J. (1980). Gastropod growth rate, allometry, and adult size: environmental implications. In *Skeletal growth of aquatic organisms: biological records of environmental change* (ed. D. C. Rhoads and R. A. Lutz), pp. 379-394. Plenum Press, New York.

Von Bertalanffy, L. (1933). *Modern theories of development*. Oxford University Press, London.

Von Bertalanffy, L. (1952). *Problems of life*. Watts and Company, London.

Waddington, C. H. (1947). *Organisers and genes*. Cambridge University Press, Cambridge.

Weber, B. H. & Depew, D. J. (1996). Natural selection and self-organization: dynamical models as clues to a new evolutionary synthesis. *Biology & Philosophy* **11,** 33-65.

Webster, G. & Goodwin, B. C. (1982). The origin of species: a structuralist approach. *Journal of Social and Biological Structures* **5,** 15-47.

Westermann, G. E. G. (1966). Covariation and taxonomy of the Jurassic ammonite *Sonninia adicra* (Waagen). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* **124,** 289-312.

Wolpert, L. (1969). Positional information and spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25,** 1-47.

Yeap, K. L., Black, R. & Johnson, M. S. (2001). The complexity of phenotypic plasticity in the intertidal snail *Nodilittorina australis*. *Biological Journal of the Linnean Society* **72,** 63-76.

Zhang, Y., Xie, L. P., Meng, Q. X., Jiang, T. M., Pu, R. L., Chen, L. & Zhang, R. Q. (2003). A novel matrix protein participating in the nacre framework formation of pearl oyster, *Pinctada fucata*. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **135,** 565-573.

"Cell and tissue, shell and bone, leaf and flower, are so many portions of matter, and it is in obedience to the laws of physics that their particles have been moved, moulded and conformed… Their problems of form are in the first instance mathematical problems, their problems of growth are essentially physical problems".

D'Arcy Thompson (1952, p.7)

Part I:

Evolution and development:

problems and prospects

Chapter 1 - Models of morphogenesis: past, present and very next future

Reference: Urdy, S. Evo-devo of morphogenetic models. *submitted*.

Abstract

Since the 19th century, the most recurrent and intriguing debates of evolutionary biology eventually focus on the origin of biological variability and regularity. Historical and law-like explanations appear in continuous tension since that time and such controversies are to be found at the developmental level as well. Nowadays, the generation of biological forms seems to be questioned (and therefore answered) in relatively distinct ways that can be highlighted by the researchers' semantic preference for the terms patterning, pattern formation and morphogenesis, respectively: 1- the first view concentrates on how cells spatiotemporally differentiate and proposes that cells can interpret thresholds along monotonic morphogens gradients in order to specify a unique positional value with respect to some boundaries (positional information). The interpretation of thresholds is assumed to depend on the cells' developmental history and genetic background; 2- the second view focuses on the emergence of spatiotemporal heterogeneities (mainly chemical gradients) and stresses the role of self-organizing dynamics in the establishment of chemical morphogenetic fields; 3- the third view rather places emphasis on how three-dimensional shape comes about with embryonic cell movements and explores the role of physical forces (mainly continuum mechanics but also cell-cell interactions and mechano-transduction) in the origination of biological form. I investigate how each of these three views relates to the fashionable concept of self-organization, which is still waiting for a formal undiputable definition in other respects. I argue that the distinction between pattern formation and morphogenesis, relying on the hypothesis that the underlying mechanisms are respectively chemical or mechanical in nature is misleading. 'Generative structuralism' is revisited in regard to the conceptualization of development and evolution. This review ends with a short overview of the state of the art in modeling morphogenesis. Some emerging trends, in accordance with 'generative structuralism', are discussed.

Key words: model – morphogenesis – positional information – reaction-diffusion – mechano-transduction – self-organization – structuralism – generic physical properties – evo-devo.

Contents

I. Introduction

Over the last few decades, developmental biology has offered tremendous progress in the identification and analysis of a large number of genes involved in the regulation of developmental processes. However, achieving an integrative view of how this wealth of genetic data affects morphogenesis and its evolution is challenging (e.g. see Nijhout, 1990; Gilbert & Sarkar, 2000; Murray, 2000; Goodwin, 2000 for diverse emphases).

Various conceptual difficulties have been pervading the debate on development and evolution of organisms for centuries, the most recurrent one probably focusing on the causal explanation of organismic variability and regularity. Already during the pre-Darwinian time, the debates were revolving around the functionalist/structuralist dichotomy (see Amundson, 2005 for a revised history of the 19th century concerns). Regardless of various theological assumptions, researchers were divided on a critical question: are coincidental functional needs sufficient to explain the regularities observed among groups of organisms ('Unity of type', homologies in modern terms)? Feeling that the answer to this question could be negative, some researchers developed a structuralist approach to morphology and embryology that turned useful in building and reifying the 'Natural System'. In that way, structuralists contributed importantly to one crucial aspect of Darwin's work: the recognition of the genealogical relationships between species.

Before the end of the 19th century, embryologists mainly thought that heredity was located in the cytoplasm and some of them remained skeptical about the chromosomal basis of heredity until 1920 (like William Bateson, see Horder, 2001). But the rediscovery of 'Mendelian laws' lead to the redefinition of the heredity concept in terms of genetics. Thus, it replaced the 19th - early 20th century 'epigenetic view' according to which heredity was a matter of embryological development.

The divorce of heredity from embryology was indeed timely to shape evolutionary theory. By assuming particulate inheritance of morphological characters, the phenotype could be equated to the genotype, an equivalence that was especially instrumental in bringing about the 'Modern Evolutionary Synthesis'. Initiated in the 1930's and relying on the null hypothesis model of Hardy-Weinberg developped in 1908, the 'Modern Synthesis' assumed that phenotypic variation was 'random', at least with regards to selection.

In order to be initiated, the 'Modern Synthesis' had to consider development as a 'black box'. It was assumed that: (**1**) - genetic variation for most traits was existent in populations; (**2**) - the relationship between genotype and phenotype was simple (linear); (**3**) - phenotypic variation was small and gradual (Salazar-Ciudad, 2006a). Under these assumptions, embryology was seen as largely irrelevant to evolution and some claimed that selection alone was sufficient to explain the evolutionary process (Fisher, 1930).

For most embryologists of the time, the 30's and the initiation of the 'Modern Synthesis' mark the first clear definition of the place of genetics within biology (Horder, 2001). After the Second World War, the classical problems of experimental embryology ('Spemann school') become old fashioned. It has been realized that attempts at understanding the causal mechanisms of development under the joint concepts of 'organizer' and 'morphogenetic field' were premature (e.g. Waddington, 1956, and see

below). Consequently, most of the researchers involved in this field turned their attention to more manageable problems (see Horder, 2001). The structuralist program, primarily concerned with the building of a theory of form was progressively abandoned by embryologists[1]. They had to provide first a thorough description of the phenomena involved in development before any such attempts could be judged realistic (Waddington, 1956). So, from the 40's, embryology got progressively fragmented into cell biology and molecular biology (Horder, 2001). As a consequence, the connection between evolution and development waned. Also, most embryologists of the time, rejecting the new definition of heredity did not participate to the 'Modern Synthesis' (notable exceptions are Gavin de Beer, Julian Huxley and Thomas Morgan, see Horder, 2006).

The functionalist/structuralist controversy was temporally weakened whilst the dominant view assumed that adaptation by natural selection was the 'ultimate' cause for homologies and homoplasies, which were therefore viewed as mere products of the history of adaptation. This quasi-exclusive historical/contingent explanation of biological order became so widespread that few authors opposed it during this period (but see for example Waddington, 1947; 1977). The 'radicalization' of Neo-Darwinian positions with regards to development became particularly strong after a series of influential philosophical books. For instance, Mayr (1961, 1963) introduced a dichotomy between 'proximate' and 'ultimate' causes of variation[2], the former being restricted to the ontogenetic time scale and the latter being reserved for the evolutionary time scale. In this view, development was relegated to the mechanistic ('proximal') cause of ontogenetic variation while natural selection was understood as the historic ('ultimate') cause of evolutionary modification. As noted by Love (2003, p. 335), "*Mayr's distinction between ultimate and proximate causation affords him a ... rationale for why ontogenetic studies cannot contribute to evolutionary theory*".

Although largely dismissed, the form/function dichotomy persisted across the 'Modern Synthesis' and the deep-seated conflict between functionalist and structuralist interpretations reawaken in the late 70's - early 80's, as among others, embryology and morphology were reconnected to evolutionary questions (Gould, 1977; Alberch, 1980; Raff & Kaufman, 1983; and see review by Gilbert, 2003). The pragmatic bracketing of development operated by the 'Modern Synthesis' (which was certainly a useful working hypothesis in the 40's) became more and more questioned. Some authors repeatedly argued for the search of general rules of development advocating that at least some amount of biological order was caused by the

1 Many reasons for this *"disillusioned collapse"* have been suggested (Horder, 2001, p. 110; see also Gilbert, 2003). Among them, one can suggest that analyses of the chemical nature of fields discouraged many efforts. Moreover, methods of the time (mainly grafting experiments) were not able to shed light on contradictory experiments suggesting that sometimes development could be understood as mosaic or as regulative. (Note that these two difficulties are still far from resolved nowadays). Also, the wholist and vitalist influences under which studies of experimental embryology have been conducted in the 19th century and early 20th century have been widely discounted from the 40's (e.g. by Schrödinger, 1944 in his book 'What is Life'; see also Gilbert & Sarkar, 2000 for a discussion of the 'bad company' of organicism). Reductionism (atomism) became popular and *"explanations for biological phenomena were sought in the 'cellular biology' and then increasingly in 'molecular biology'"* (Horder, 2001, p. 111). The subsequent overlying rise of genetics justified the success of the reductionist (atomist) approach. (Note also, that although there are some ingredients of truth in this statement, many authors extensively used this argument in perfect circularity from the 70's to defend a drastic gene-centered view of development and evolution and sometimes to justify the "*failure of the structuralist program"* (Mayr, 1963). It contributed to the marginalization of higher level approaches to development, although they were not necessarily 'anti-reductionist' (but rather anti-atomist).

2 In Mayr's terminology, 'proximal causes' refer to the material and efficient causes in Aristotle's terminology, while '**ul**timate causes' refer to the formal and final causes in Aristotle's terminology.

dynamics of development (Gould & Lewontin, 1979; Alberch, 1980; Webster & Goodwin, 1982; Alberch & Gale, 1985; Goodwin, 1988; Oster *et al.,* 1988; Alberch, 1989; Kauffman, 1993). The debate concentrated mainly on the nature and clarification of the concept of constraints (Alberch, 1980; Maynard-Smith *et al.,* 1985) and the specific role that one has to attribute to natural selection and development respectively to account for the origin of order (Kauffman, 1993). Unfortunately, the debate on constraints produced more heat than light; one reason for it can be found in the different conceptions of the ambiguous term constraints that functionalists and structuralists endorse (see Amundson, 1994 for a highlighting comment on this issue). Also, it was recently reminded (Salazar-Ciudad, 2006a) that the selection/developmental constraints debate relied on two different assumptions about the relationship between genotype and phenotype (linear/non-linear) and what kind of morphological variation is produced by development (gradual/discrete, unbounded/limited).

The 90's have been marked by the successful overwhelming launch of developmental molecular genetics, and its hope of finally tracking the developmental origin of morphological variation down to DNA. The Human Genome Project, achieved in 2000 can be viewed as a caricature of this 'dream', as Lewontin (2000a) puts it, a project often depicted as the search for the Holy Grail in commercial contexts. But genetic studies revealed an unsuspected amount of conservation of genes and developmental pathways among distantly related organisms (Gerhart & Kirschner, 1997; Duboule & Wilkins, 1998). Once again, the explanations of conservation of 'body plans' and morphological variation observed among them were not yet at hands. As put by Goodwin (2000, p. 16), *"for what we seem to be faced with is a basic disjunction: neither historical narratives linked to genetic change, nor a detailed understanding of molecular composition, can explain the emergent morphological order observed at the level of organisms nor the logical (i.e. intrinsic) relationships between different types of organism that make taxonomy possible"*.

However, molecular studies, and especially those focusing on homeotic mutations, revived the discussion about the relationship between development and homology, through the revelation of deep molecular homologies among different developing structures in organisms and between distantly related organisms as well (e.g. Gilbert, Opitz & Raff, 1996; Hall, 2000; Gilbert & Bolker, 2001; Hall, 2003). Extensive developmental studies also led to the more and more explicit 'rediscovery' of the complex relationships between genotype and phenotype, providing the impulse for biology to enter into the 'post-genomic' era as many claimed.

Several authors are now waiting for a 'revolution', one in which new conceptual tools and methodologies will allow the understanding of the complexity of living organisms while evading genetic reductionism (Strohman, 1997). This echoes Waddington's remark (1947, p. 148) some 50 years ago according to which *"[t]he developmental side of biology - embryology, genetics and evolution - seems to be reaching a point where radically new types of thinking are called for"*. Certainly, complexity sciences raise many conceptual and methodological challenges which are largely incompatible with the linear way of thinking of the central dogma of molecular biology on one side, and the atomization and particulate inheritance of morphological characters assumed by the 'Modern Synthesis' on the other side.

The integration of different views on development and evolution (provided that it is indeed possible) obviously requires inputs from many different research fields, encompassing biological, chemical, mechanical and mathematical approaches, as many authors already pointed out. This argument is hardly new since such approaches to morphology and morphogenesis can be traced back to His (1888), D'Arcy Thompson (1952) and Turing (1952). Moreover, journals particularly devoted to theoretical aspects of biology exist since 50 years. Thanks to computers, the use of the differential equations to the 'problem' of morphogenesis flourished from the 80's. More recently genetics and developmental journals devoted special issues to the modeling approaches of development and morphogenesis (e.g. Stemple & Vincent, 2004; Beloussov & Gordon, 2006). However, with the fragmentation of embryology from the early 20th century into research fields using different methods of investigation and explanation, some problems regarding the conceptualization of development still need to be addressed. In this paper, I will attempt at discussing some of them.

II. The conceptualization of embryogenesis in the 50's

The earliest attempts at understanding embryology in causal terms are generally attributed to Wilhelm Roux, who coined the term 'Entwicklungsmechanik'[3]. At that time, the concepts under which experimental embryology was working were slowly emerging and the problematic was consequently loosely defined. But since the late 1950's, research on development has been mainly driven under four relatively distinct sets of problems, namely cell differentiation, pattern formation, morphogenesis and growth.

The first set of problems is concerned with *"the gradual change in the nature of a mass of living matter, which may consist of a part of a cell or more usually of a group of many cells"* (Waddington, 1956, p. 11). Differentiation characterizes the phenomenon by which cells in the embryo progressively acquire more specific characteristics. For instance, the columnar cells of the ectoderm can acquire the characteristics of nerve cells, with are 'star' shaped and possess an axon; or the cuboidal cells of mesoderm can acquire the characteristics of muscular cells, which are elongated and filled with contractile muscle fibers. Thus, cell differentiation generally leads to a change in cell shape and in the products synthesized by the cells (cell's phenotype). There exists a limited number of different cell types (about 200 sorts, see Kauffman, 1993). Differentiation deals with the processes that make cells acquire different sets of properties among a definite set of possibilities (usually, cells of the distinct embryonic layers only have the possibility to differentiate into a subset of these possibilities). The neoplastic transformation of cells (cancerous cells) is also a problem of differentiation since these cells acquire new properties (e.g. previously tightly bound epithelial cells can become motile).

To distinguish differentiation from the types of phenomena that produce tissues and organs, Waddington (1956, p. 415) introduced the term 'individuation'. He noted that 'individuation' has two rather different aspects: pattern formation and morphogenesis.

3 Some interesting attempts can be dated back to Wilhelm His (1888), but it seems that his approach at simulating the folding of the neural tube with rubber paste were not taken very seriously by contemporary researchers. Horder (2001, p. 131) says that *"Wilhelm His's early emphasis on mechanical forces in the embryo may, due to the almost caricaturing effect of his modeling approach, actually have inhibited later serious interest"*.

Pattern formation is synonym with the *"the term 'regionalization' [which refers] to the appearance of different parts within an originally uniform expanse of tissue"* (Waddington, 1956, p. 415). A classical example is the formation of the eye spot pigment patterns in lepidopteran wings, where cells in different regions of the wing secrete distinct pigments. Similarly, the formation of patterns of venation in *Drosophila* wing is also viewed as a case of pattern formation: some cells will be retrieved to form veins, whereas others will not. The formation of the five ray pattern of cartilaginous rudiments in the digit plate of tetrapods is a most cited example of pattern formation in modern developmental biology textbooks.

The second aspect of 'individuation' is morphogenesis *sensus stricto*, *"the moulding of a mass of tissue…into a coherent structure which is recognized as having some unitary character of its own, which is usually acknowledged by giving it a name as an anatomical organ"* (Waddington, 1956, p. 12). The most obvious example of morphogenesis is gastrulation, which is characterized by the invagination of the blastoderm followed by the movement of cells toward the interior of the embryo along the blastoderm. Another example is the folding of the neural plate which gives rise to the neural chord, rudiment of the future nervous system of vertebrates.

As noted by Waddington (1956, p. 12), these three sets of problems (differentiation, pattern formation and morphogenesis *ss*) are indeed closely interwoven. For example, after gastrulation, the central part of the vertebrate embryo become characterized by neural crest cells (future nervous cells), whereas on the left and right sides segmented regions of future muscle cells are being formed (somites). But this 'regionalization' of cell types involves the folding of the neural plate and the migration of fibroblasts. Nevertheless, Waddington (1956, p.12-13) notes that *"it is important to disentangle them from each other, since each requires a different category of explanation. Differentiation could be a purely chemical process, involving nothing more than changes in substances…Regionalization, on the other hand, involves some references to a spatial framework; it requires at least physico-chemical notions, such as diffusion, crystallization or the like. Finally, the moulding of a mass of material into a shape, as in morphogenesis, can only be brought about by the operation of forces, and thus requires a discussion in terms of physics"*. He goes on to say (p. 438) that *"[c]learly the pattern formation and the morphogenesis are inextricably involved with each other, and can only be separated conceptually by roughly classifying some of the events as rather more chemical in nature (and therefore related to pattern formation) and others as more definitely physical (and therefore connected with morphogenesis)"*.

Waddington (1956, p. 415) categorizes differentiation, pattern formation and morphogenesis *ss* as "primary morphogenesis" since these terms describe the phenomena *"by which the original shape of the organ rudiments is first brought into being"*. Growth is understood as "secondary morphogenesis" since *"the shape of organs and of body as whole continue to change throughout most of life owing to the unequal growth of different parts"*. His favorite example (Waddington, 1956, p. 295-296) is that of the final shape of the *Drosophila* wing, which he showed to depend *"not only on successive phases of cell division and cell expansion but also on deformations of the whole structure resulting from the changes in pressure of the body fluid contained in it"*. Other classical examples concern the

inter-species transplant experiments of whole organs growing at different rates in different species performed in the 30-40's. For instance, the transplant of the eye cup of the salamander *Amblystoma triginum* to *Amblystoma punctatum* or *vice versa* has revealed that the eyes retain their own characteristic size regardless of that of the host. However, when one of the two main elements of the eye of one species (eye-cup and lens) is combined with the other element of the other species, the final relative sizes of the two elements are nearly adjusted to each other.

The four set of problems defined by Waddington (1956) are still strongly underlying much of the recent research in developmental biology. But the separation between these four sets of problems has been considerably strengthened over the years. Waddington's categorization essentially appears as a pragmatic and scientifically progressive approach of embryogenesis, trying to provide more coherence to a field which was puzzled by the complexity of the organism. For instance, he writes: *"[i]t is only in recent years, as our understanding has increased, that the distinction between these types of phenomena has become important for experimental embryology. The greater part of the subject has been developed in terms of more loosely defined notions, which have in practice been closer to the idea of differentiation than to the other two concepts [pattern formation, morphogenesis]"* (Waddington, 1956, p.13).

Nowadays, it is often literally assumed that different processes underlie pattern formation and morphogenesis *ss*: cell-cell signaling for the former and physical properties of cells and tissues for the latter. This assumption allows morphogenesis *ss* to be circumscribed to particular spatial and temporal scales. Typically, it is believed that pattern formation is characteristic of early development for gene expression patterns are sometimes observed before obvious cell growth or cell movements; or early specification of cell fate is assumed to precede the 'main' morphogenetic events. An emblematic example is the vertebrate limb: since grafting experiments point out that the specification of cell fates in the limb bud could precede mesenchymal cell condensations, it is believed that physical forces acting on cells and within the limb bud are only worth being studied in later stages of development (Wolpert & Hornbruch, 1990).

However, relatively recent developments in biophysics and tissue engineering point out that cell differentiation, cell shape, cell spatial arrangement, cell movements, cell growth and cell death are dependent on the physical state of cells and tissues *in vitro*, *in vivo* and *in silico* (e.g. Ingber, 2002; Nelson *et al.*, 2005). Physical forces have been shown to affect in striking ways phenomena that were mainly thought as being only (or mainly) under the control of cell-cell signaling (cell differentiation, pattern formation, growth, apopotosis). Since more than thirty years, an accumulation of experimental evidence has highlighted that the physical state of cells and tissues could also influence gene expression. This is known as mechano-transduction: *"how the cells sense mechanical forces and convert them into changes in intracellular biochemistry"* (Ingber, 2006a, p. 256). Considerable progress in the understanding of mechano-transduction has been achieved during the last fifteen years (for reviews, see Murray, 2000; Brouzés & Farge, 2004; Ingber, 2006a & b; Wang & Thampatty, 2006).

First, I will depict three relatively distinct ways to question the generation of biological form; three views that are superficially typical of different research field. This is highlighted

by the researchers' semantic preference for the terms patterning, pattern formation and morphogenesis. What are the questions underlying these terms? Which hypotheses are put forward? Differentiation and growth will only be briefly discussed with respect to mechano-transduction, in the last two sections of this paper. But the arguments developed throughout this paper would equally apply to differentiation and growth.

Second, I will investigate how each view situates itself with respect to the 'self-organization' concept, a catchword continuously escaping attempts at formally defining it. This will help me to draw some similarities and differences between the three views I sketch.

Third, I will revisit the 'generative structuralism' framework in regard to the conceptualization of development and evolution. I will end this review by drawing a short (and user-friendly, I hope) overview of mathematical models of morphogenesis *sl* (Waddington's individuation).

III. Modern conceptualization of development in three research fields

My goal is to bring together studies that are rarely discussed together so that some important differences in concepts may be overlooked. I will identify each view by the 'research fields' in which they are most commonly found, that is to say, 'experimental developmental biology', 'theoretical developmental biology' and 'developmental biophysics'. These categories are useful for my purpose and should not be intended as either sharp or easily recognizable ones nor do they imply that all scientists who could be classified in a one of these fields would necessarily homogeneously approve the mainstream view I sketch (indeed many authors may belong to several categories). Somehow, 'semantic convergences', tied to sloppy definitions, blur the differences in the questions addressed and the hypotheses put forward.

Table 1 summarizes the central questions and hypotheses that in my opinion underlie the terms patterning, pattern formation and morphogenesis as commonly understood in the three research fields that mainly focus on one of these questions. Using vertebrate limb development as an example, Figure 1 summarizes the three main views that will be discussed in this section and Table 2 provides examples of corresponding types of explanations.

(1) Patterning of 'experimental developmental biologists'

Most 'experimental developmental biologists' retain the conceptualization proposed by Waddington (1956) and many efforts have been devoted to the understanding of the molecular mechanisms underlying pattern formation.

Pattern formation is defined as *"the process by which the spatial aspect of cellular differentiation is organized; how, for example, cartilage and muscle differentiate in just the right place during the development of the limb. Pattern formation, thus, focuses less on cell differentiation itself. After all, the difference between our arm and that of a bat, seal, or hippopotamus is not due to differences in muscle and cartilage differentiation but to their spatial pattern and later growth",* (Wolpert, 1996, p. 359). This definition has been subsequently refined to include a temporal dimension and has been extended beyond patterns of cell differentiation: *"Pattern formation is the process by which a spatial and* ***temporal*** *pattern of* ***cellular activities*** *is organized within the embryo so*

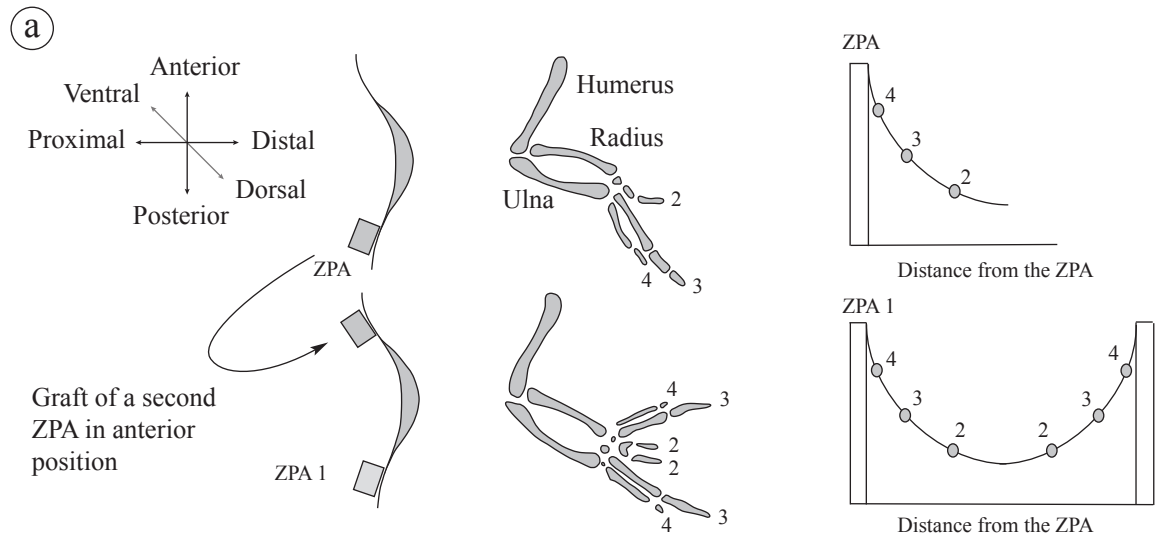
	Question	Hypothesis
Patterning	How do spatial and temporal patterns of cell differentiation are coordinated/ regulated/specified?	Monotonic morphogens gradients can provide cells with positional information
Pattern formation	How do chemical heterogeneities can emerge from homogeneous initial conditions?	Reaction-diffusion models can explain the self-organization of molecular gradients
Morphogenesis	How does shape explicitly come about?	Physical forces, cell-cell interactions and mechano-transduction can explain the morphogenetic movements and influence differential growth

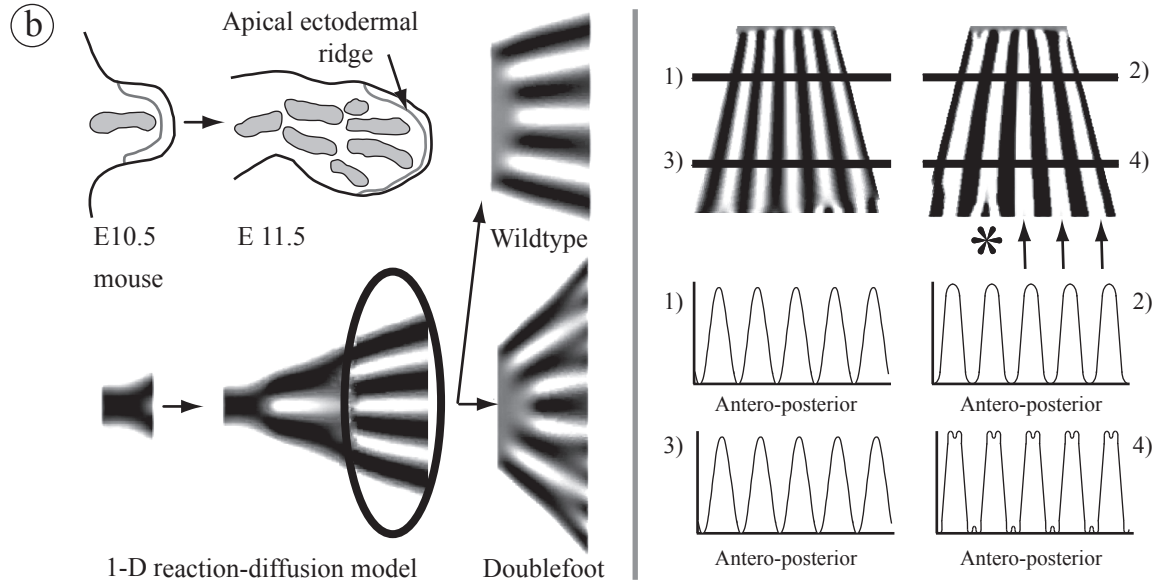
Table 1: Summary of the central question and hypothesis underlying the terms patterning, pattern formation and morphogenesis.

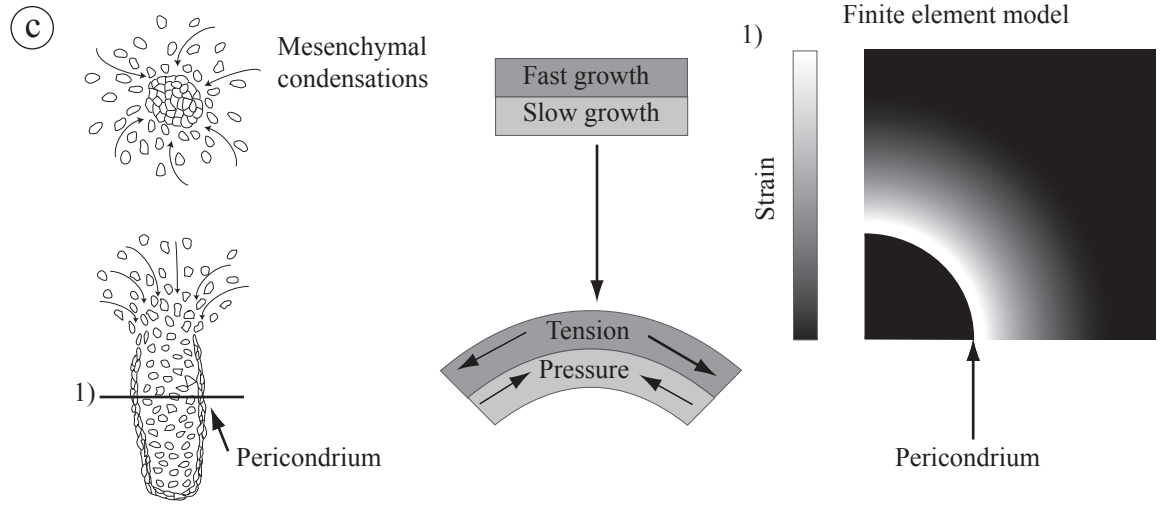
Example	Vertebrate limb
Patterning	"One possible extension of the early specification model is that cells in the limb bud have already acquired not only proximo-distal positional values but also antero-posterior and dorso-ventral positional values. At the other extreme, building on the progress zone model, one could envisage that cells acquire all their positional values over time as the limb bud grows out". Tickle 2003, p. 450
Pattern formation	"The activator-inhibitor mechanism described above allows the generation of graded concentration profiles that can instruct cells about their positions in a field. In other words, it allows the generation of positional information". Meinhardt and Gierer 2000, p.757
Morphogenesis	"Although diffusible molecules can act over a distance, the distance and rate of signal propagation are limited. In contrast, tensions and pressures created at one site of an organism may impose tensions and pressures at another site, simply due to the physical connections that are made between anatomical structures. In addition, the propagation of these signals could be virtually instantaneous. Therefore, differential growth in one portion of the developing limb has the potential to create growth generated stresses over the entire limb and may influence development and morphogenesis at distant locations". Henderson and Carter 2002, p. 647

Table 2: Example of the type of explanations used to account for vertebrate limb development in the research fields associated with patterning, pattern formation and morphogenesis respectively.

Figure 1: **a-** Positional information is used to account for duplicated digits in chick embryo when a secondary ZPA (zone of polarizing activity) is transplanted to the anterior side of the limb bud. *Right*: mirror-image of morphogen concentration and thresholds corresponding to digits. Redrawn from Wolpert *et al.*(2006). **b-** *First column*: one-dimensional reaction-diffusion model on growing domain to account for the proximo-distal emergence of cartilaginous elements in the mouse limb and the phenotype of *Doublefoot* mutant. *Second column*: the same model is used to simulate the formation of 'thin-digits', sometimes found in *Doublefoot* mutants. The spatial concentration of the activator from which the pattern of digits is derived is shown. *Arrows*: 'thin-digit'-like peaks of activator. *Star*: 'thin-digits' can become 'normal thickness'. Redrawn from Miura *et al.* (2006). **c-** *First column*: mesenchymal condensations in the limb bud illustrating the formation of the perichondrium. *Second column*: diagram illustrating the strains generated in connected tissues that grow at different rates. The faster growing tissue experiences tension whereas the slower growing tissue is under pressure. *Third column*: cross-section of a condensation illustrating the strains generated by the rapid growth of the condensation compared to the surrounding cells. *White*: tensile strain. *Black*: compressive hydrostatic stress. Growth of the condensation generates mechanical simuli which may influence cell and matrix biology and are correlated with the genes characteristics of chondrogenesis. Redrawn from Oster *et al.*, (1988) and Henderson *et al.,* (2007).


a
Anterior
Ventral
Proximal
Distal
Dorsal
Posterior
ZPA
Graft of a second ZPA in anterior position
ZPA 1
Humerus
Radius
Ulna
2
4
3
4
3
2
2
4
3
ZPA
4
3
2
Distance from the ZPA
ZPA 1
4
3
2
4
3
2
Distance from the ZPA


b
Apical ectodermal ridge
E10.5 mouse
E 11.5
Wildtype
1-D reaction-diffusion model
Doublefoot
1)
2)
3)
4)
*
1)
2)
Antero-posterior
Antero-posterior
3)
4)
Antero-posterior
Antero-posterior


c
Mesenchymal condensations
1)
Pericondrium
Fast growth
Slow growth
Tension
Pressure
Finite element model
1)
Strain
Pericondrium

that well ordered structure develops" (Wolpert *et al.,* 2006, p. 16, my emphasis). The question of pattern formation in developmental biology is more or less explicitly framed with respect to the hypothesis proposed more than 30 years ago by Wolpert (1969): the positional information concept.

This hypothesis intimately binds pattern formation with instructive metaphors: "*In the developing arm, for example, pattern formation is the process that enables* ***cells to 'know'*** *whether to make an upper arm or fingers, and where the muscles should form"* (Wolpert *et al.,* 2006, p. 16, my emphasis). The concept of positional information seems so widely accepted that some claim that it "*changed the way we think about pattern formation in the embryo and allowed new generations of molecular developmental biologists to frame their questions in a way that would give sensible answers*" (Smith & Wolpert, 2000, p. 85). And indeed the question is generally summarized as follows: "*Patterning is the mechanism that coordinates cell position, proliferation and differentiation during embryogenesis, and is mediated by cell signaling interactions, of which morphogen gradients are of key importance. This complex mechanism ensures that characteristic features such as the head, limbs, nervous system, organs, and even individual cartilage and bone elements, such as the vertebrae, develop in the correct location with the appropriate size and shape. One of the major questions in developmental biology is how cells acquire positional information*" (Trainor, 2003, p. 5562).

In this quotation, and more generally in developmental biology, it is not clear whether the term 'patterning' is used as a short-cut for pattern formation (designating a phenomenon) or whether it is understood as a (molecular) mechanism (tied to positional information or not) that account for the phenomenon of pattern formation. I suspect that patterning is usually equated with morphogens action and/or patterns of genes that somehow predict the spatial location of future structures (like cartilage rudiments in limb bud development, or presumptive tooth cups in dental formation). It is also worth noting that *"[t]he phrase "positional information" is now deeply embedded within the common vocabulary of developmental biologists. It is used frequently and unquestioningly; it is rarely explicitly attributed to Wolpert or his model. .... The term is now, one suspects, sometimes used in a loose and general sense, not to imply any model at all, but merely to refer to the "problem of development" itself, i.e. how cells know how to become different"* (Horder, 2001, p. 117).

If one strictly follows Wolpert's scheme, the positional information concept implies a spatial coordinate system that cells can access to specify their unique positional value with respect to some boundaries defining a developing system (field). Monotonic gradients of soluble molecules, called morphogens are supposed to be established while such substances diffuse in the external cell milieu from their source of synthesis (the so-called organizer). Such concentration gradients are assumed to provide a coordinate system leading to thresholded responses of cells (Fig. 1a). Pattern formation is thought to be due to two independent processes. Cells first assess their positional value in a concentration dependent manner between thresholds (e.g. dorsal-ventral or proximal-distal) then they interpret this information and differentiate specifically according to their genome and developmental history.

This two step process implies that positional information is not specific to any

developmental field, and indeed it has been proposed to be universal (Wolpert, 1969). However, it also implies that what happens in a developing system (field) must not alter (through feedback) the morphogenetic gradients which provide positional information in the first place.

In developmental biology, morphogenesis is defined as the *"moulding of form—[and with it] the emphasis is on the forces bringing about changes in shape*" (Wolpert, 1969, p. 2). In general, morphogenesis is assumed to result from the specifically induced cell behaviors (i.e. mitosis, apoptosis, migration, adhesion, etc) resulting themselves from the specific interpretation of positional information. Morphogenesis is thus treated as a slave process, and in practice this part of the question is left quasi-untouched. Admittedly, molecular patterning (morphogenetic signals and more widely cell-cell signaling) and morphogenesis (other cellular behaviors and forces) are tightly interwoven processes, but in practice they are treated as rather distinct issues. It seems that the effective understanding of how morphogenetic movements are effectively generated is at best not a priority. At worst, it is assumed that correct interpretation of positional values conferred by morphogens ensures that the organisms' tissues "*develop in the correct location with appropriate size and shape*" (Trainor, 2003, p. 5562).

(2) Pattern formation of 'theoretical developmental biologists'

A different perspective is endorsed by 'theoretical developmental biologists' whose view of pattern formation is deeply concerned with the problem first addressed by Turing (1952): could chemical heterogeneities arise from random perturbations of an initially uniform concentration? This view shares with the developmental biology's stance the dominance of the chemical level of investigation and the separation of the question of pattern formation from the one of morphogenesis *ss*. However, this view of pattern formation implies a dynamical view of chemical signaling deeply rooted in non-linear non-equilibrium equations.

The most widely proposed and studied model to account for pattern formation, namely reaction-diffusion, has even become paradigmatic of the self-organization concept since Gierer and Meinhardt (1972) proposed *'short range activation and long range inhibition'* as a theory of biological pattern formation. Such models belong to the class of pre-pattern mo-dels, in the sense that the pattern of chemical concentration (or alternatively pattern of gene expression) somehow foretells the location of the future morphological pattern. For instance, in the developing epithelium of vertebrates, the areas of higher chemical concentration would correspond to positions where the scales / feathers / hair will form.

The original Turing model (1952) was concerned with the emergence of spatial heterogeneities (the so-called Turing structures) by means of two interacting substances (that he called morphogens) diffusing in the external milieu and exhibiting antagonistic behavior: one molecule is called activator and is assumed to exhibit autocatalytic kinetics and to stimulate the synthesis of an other molecule called inhibitor that counter-acts activator synthesis by consuming it. Diffusion is generally thought of as a homogenizing process. But in combination with two coupled antagonists reactions (autocatalysis and inhibition), a stable homogeneous concentration of molecules can be destabilized by diffusion in response to small random perturbations so that stable heterogeneous patterns of

concentration would emerge. This phenomenon is called 'diffusion-driven instability'. According to the relative diffusion range of the activator and inhibitor, the initial perturbations of the stable homogeneous solution can be smoothed out or amplified. If so, the whole system stabilizes to another predictable equilibrium state (e.g. spatial patterns, temporal oscillations or traveling waves if one considers the interaction between three morphogens) independently of the initial perturbations[4].

Indeed, only opposing reacting kinetics of two molecules are necessary for their concentration to oscillate over time. This is exactly the same generic process that has been proposed to be involved in the well known Lotka-Volterra predator-prey models. One has to assume that the more preys, the more predators (predators number increases when plenty of food is available) but the more predators, the less preys (since predators also consume more preys). Such a mechanism can give rise to temporal patterns of varying number of preys and predators (at one moment, there is more preys than predators then the situation reverses).

To the contrary, the emergence of 'Turing structures' (spatial patterns, Fig. 1b) generally requires that inhibitor diffuses more rapidly than activator, so that the activator concentration does not smooth out all over the domain. This hypothesis is more generally known as *'short range activation and long range inhibition'* (Gierer & Meinhardt, 1972) or *'local self-activation and lateral inhibition'* (Meinhardt & Gierer, 2000). Many theoretical systems may exhibit such properties and they do not necessarily imply chemical diffusion (e.g. neuronal models, competition models, etc).

Reaction-diffusion models have been proposed to be at work in a variety of contexts: mammalian coats marking (Bard & Lauder, 1974; Murray, 1989; Goodwin, 1994), butterfly wing patterns (Nijhout *et al.,* 2003), zebrafish pigmentation patterns (Barrio *et al.,* 1999; Yamaguchi, Yoshimoto & Kondo, 2007), molluscan shell pigmentation or ornamentation (Meinhardt, 1995; Hammer & Bucher, 1999), chondrogenesis in tetrapod limb (Newman & Frisch, 1979; Benson, Maini & Sherratt, 1993; Miura & Maini, 2004; Miura *et al.,* 2006), tooth formation (Maini, 1996), *Acetabularia* whorl formation (Harrison, Wehner & Holloway, 2001), plants phyllotaxis patterns (Meinhardt, 2003) and patterns of gene expression, as those involved in *Drosophila* segments and imaginal disks (Kauffman, Shymko & Trabert, 1978; Bunow *et al.,* 1980; Nagorcka, 1988) or vertebrate notochord formation (Meinhardt, 2000).

(a) Different concepts of morphogens and morphogenetic fields

Reaction-diffusion models have been repeatedly proposed as a mechanism explaining the emergence of monotonic Wolpertian morphogens gradients (e.g. Meinhardt & Gierer, 2000, see also source-sink models, e.g. Gierer & Meinhardt, 1972) and signaling cascades or gene expression patterns as well (Schiffmann, 1991; Miura & Shiota, 2000; Miura & Shiota, 2002).

Wolpertian morphogens are defined as soluble substances which diffuse from their source and thus give rise to a uniform concentration gradient (Fig. 1a). But the inevitable question is: where does the source of morphogens come from in the first place? Models relying on non-linear equations can propose at least an elegant answer to this question (Monk, 2000).

4 A homogeneously spatial increase of the stable concentration of activator and inhibitor suffices to destabilize the system for example.

As Wolpertian morphogens are supposed to induce dose dependent responses, the second question is: how do cells detect sharp thresholds along monotonic gradients? Some hypotheses have been proposed to account for the interpretation of morphogens gradients that do not depend on thresholds (Meinhardt, 2000). This release of the Wolpertian positional information concept is achieved by a dynamic interaction among target genes.

'Theoretical developmental biologists' also proposed some hypotheses to increase the robustness of reaction-diffusion models (e.g. Maini, 1996; Crampin, Gaffney & Maini, 1999) while many more investigated the effect of the domain size or shape on the resulting pattern (e.g. Murray, 1989; Goodwin, 1994; Nijhout *et al.,* 2003; Hammer & Bucher, 1999) and the effect of various initial and boundary conditions (e.g. Dillon *et al.,* 1994; Barrio *et al.,* 1999), not to mention studies extensively combining experimental and theoretical comparisons (e.g. Yamaguchi, Yoshimoto & Kondo, 2007).

But the 'experimental developmental biologist' and the 'theoretical developmental biologist' views can only partly be equated. Morphogens, a term first coined by Turing (1952), are more widely defined in theoretical than in experimental developmental biology (e.g. Murray, 1989; Kauffman, 1993; Jaeger & Reinitz, 2006). According to the larger original definition (that of Turing), downstream regulatory genes would be understood as morphogens. To the contrary, 'experimental developmental biologists' have been searching morphogens as upstream genes regulating downstream genes. If morphogenetic gradients are monotonic, the resulting morphological pattern results solely from the cells' interpretation of the thresholds along these gradients (Fig. 1a). In other words, the morphogen concentration profile is independent of the subsequent morphological pattern. To the contrary, Turing's morphogens imply that "*the expected distribution of morphogens in space and time foretold the subsequent morphological patterns which arise. Indeed, this approach is, in a sense, a retreat to the earlier prepattern concept of Stern*" (Kauffman, 1993, Fig. 1b).

The positional information concept was originally proposed by Wolpert (1969) as a redefinition and improvement of the 1920s-1930s' morphogenetic field concept that was loosely used to describe a group of cells demonstrating regulative properties under experimental manipulations (Gilbert, 2000; Horder, 2001). This concept progressively took the form of the 'gradient field' idea, emphasizing a molecular pre-pattern that prefigures the morphological outcome (much like Turing's model). The morphogenetic field concept has been subsequently redefined by Waddington (1947) who emphasized the intrinsic dynamical properties of fields; characteristics that, according to him, should be taken into account to define the fields. Waddington (1947) stressed the regulative properties of fields and the feedback of the 'whole' (field) on the 'parts' (e.g. molecules). However, the meaning of positional information as defined by Wolpert substantially diverges from the morphogenetic field concept defended by Waddington (Goodwin, 1988; Horder, 2001; Jaeger & Reinitz, 2006 and see below). The purpose here is not to discuss the various definitions and the tortuous history of the morphogenetic field concept (and its related notions) that poses many unsolved problems. But to make a long story short, the morphogenetic field concept was of central importance to embryology in the early 20th century, was dismissed at the time of the 'Modern Evolutionary Synthesis' by embryologists turning to genetics

(such as Thomas Morgan), was subsequently revived by Wolpert in the late 60's under the umbrella of positional information and has somewhat recently regained more general attention in the Waddington's sense (see reviews in Gilbert, 2000; Gilbert & Sarkar, 2000).

The important point here is that Wolpert's view on fields is a static one, and in some way, this is also the view implied by reaction-diffusion models, insofar they are interpreted literally. Although the chemical patterns of morphogens are dynamically created, no feedback between cell behavior and chemical reactions is considered.

(3) Morphogenesis of 'developmental biophysicists'

Contrary to the views described above, morphogenesis *ss* is the real focal point of 'developmental biophysicists'. Pattern formation is given unclear or no definition at all: does it imply chemical morphogenetic gradients? Is it restricted to 2D patterns such as pigmentation? Indeed, most authors assume no dichotomy and thus consider pattern formation as part of morphogenesis: as stated by Murray (1989, p. 372):"*Morphogenesis,.., is the development of pattern and form*". In that way, the term *morphogenesis* is really close to 'Waddington's individuation (*sensus largo*). "*In the mechanochemical approach, pattern formation and morphogenesis is considered to go on simultaneously as a single process. Here the chemical patterning and the form-shaping movements of the cells and the embryological tissue interact continuously to produce the observed spatial pattern*" (Murray, 1989, p. 526).

Mechanochemical and mechanical models have been proposed in a variety of contexts, largely overlapping the classical range of application of reaction-diffusion models: epithelial and mesenchymal morphogenesis (Odell *et al.,* 1980; Murray, 1989), chondrogenetic condensation in tetrapod limb (Oster *et al.,* 1988; Hentschel *et al.,* 2004; Newman & Müller, 2005), tooth formation (Osborn, 1993), cytoskeleton dynamics (Oster & Odell, 1984; Tracqui & Ohayon, 2004), wound healing and angiogenesis (Murray, 1989; Trancqui & Tracqui, 2000), gastrulation and neurulation (Weliky & Oster, 1990; Weliky *et al.,* 1991; Davidson *et al.,* 1995; Davidson *et al.,* 1999), plants phyllotaxis patterns (Green, 1992; Douady & Couder, 1992; Green, 1999; Malygin, 2006), gastropods aperture shape and ornamentation (Morita, 1991) and *Acetabularia* whorl formation (Goodwin & Trainor, 1985).

Some authors suggested that mechanical instabilities (e.g. buckling) were the primary driver of morphogenesis. For instance, Harris, Stopak & Warner (1984) proposed to view convection driven instabilities as an alternative to chemical instabilities in the establishment of *in vitro* fibroblast condensations that were previously dissociated; patterns that strikingly resemble those appearing during feather formation or chondrogenesis. Osborn (1993) somehow provocatively proposed a *'model simulating tooth morphogenesis without morphogens'*, assuming a mechanical interaction between the epithelial and mesenchymal cell layers of the tooth germs. The different growth rates of the two mechanically coupled layers could cause them to fold into shapes determined by the forces generated with growth. In the same manner, cell rearrangements observed during gastrulation or notochord formation have been explained solely by the balance of forces between cells (Weliky & Oster, 1990; Weliky *et al.,* 1991). The effect of mechanical instabilities such as buckling has

been proposed to underlie gastrulation (Oster *et al.,* 1979; Davidson *et al.,* 1999; Keller, Davidson & Shook, 2003).

Such 'alternative' views were indeed contemporary to Turing, and for example Lehman (1957) proposed that pigmentation patterns in salamanders could be explained by assuming certain combinations of mutual attraction and repulsion between pigment cells, an hypothesis that is experimentally well supported (Olsson & Löfberg, 1993). More generally, it relates to Steinberg's differential adhesion hypothesis (Steinberg, 1970) which is itself rooted in the tissues affinities hypothesis of Holtfreter (1939). Detailed recent models of pigment pattern formation point out that the interplay of contact inhibition between different pigment cells (repulsion) and contact guidance of the extra-cellular matrix (anisotropic migration of pigment cells) associated with a permanent cell flow from the neural crest is sufficient to generate vertical pigment stripes in salamanders (Deutsch & Dormann, 2005).

In a general manner, the interplay between chemical and mechanical factors lies at the core of the 'developmental biophysicists' approach. Many authors are now able to correlate patterns of gene expression characteristics of developing cell types to the predicted patterns of stress/strains generated by cell growth and/or migration (Fig. 1c). Moreover, pathways of mecano-transduction have been identified (see for instance, Henderson *et al.*, 2007).

(a) On phenomenological models and experimental verification

Reaction-diffusion models are certainly the simplest examples of phenomenological models, which reproduce some aspects of the dynamical behavior of many unrelated systems. Some proteins have been proposed to represent Turing morphogens: the maternal gradient of *bicoid* and pair rule segmentation genes in *Drosophila* (Meinhardt, 1992), *TGF-beta2* in mouse limb morphogenesis (Miura & Shiota, 2000) and *FGF* in lung morphogenesis (Miura & Shiota, 2002), not to mention the proteins (e.g. *Dpp*, *BMPs*, *FGFs*, *Shh*) recognized as morphogens *sl* (inducing dose-dependent responses) demonstrated to be involved in various developmental contexts (for reviews see Podos & Ferguson, 1999; Vincent & Briscoe, 2001; Green, 2002; Tabata & Takei, 2004; Ashe & Briscoe, 2006; Coudreuse & Korswagen, 2006; Reeves *et al.,* 2006).

But attempts at proving the existence of true 'Turing morphogens' appear somehow futile if one considers that the hypotheses put forward by Turing and his followers were primarily driven by parsimony considerations (at least two morphogens are necessary, simple Laplacian diffusion is sufficient). Reaction-diffusion models were framed mainly to provide a heuristic answer to the emergence of spatial heterogeneities from an initial homogeneous state (which is certainly rare in real cases). Additionally, the practical necessity of considering simple reactions not too far from linearity to yield a predictive stability analysis implies in return that one has to assume a probably unrealistic difference in diffusion rates of the two morphogens in order to cross a 'diffusion driven instability'. Also, these theoretical and 'numerical' constraints imply that a quite fine tuning of parameters is necessary to find the conditions upon which 'interesting' patterns can emerge.

Many of the models of morphogenesis proposed so far share a common logical internal structure. The more general models correctly capture some of the properties of 'real' developmental systems, but from a biological point

of view they do not necessarily 'explain' the phenomenon in terms of specific mechanisms. Thus, as more experimental insights become available in a particular system, the reaction-diffusion models can not be expected to be strictly adequate, a fact that Monk (2000, p. 170) nicely recalls by quoting Thomas H. Huxley: "*[t]he great tragedy of Science - the slaying of a beautiful hypothesis by an ugly fact*".

Although the main predictions of reaction-diffusion models are generally valid, one can not infer from them a lot more than a phenomenological dynamical behavior that can be equally well reproduced by other kinds of models after all. One has to shift to more realistic and less parsimonious assumptions (necessarily derived from experimental evidence) if the models are to lead to more precise predictions, potentially driving new experiments in return (e.g. see Gursky *et al.,* 2004 in the particular case of *Drosophila* segmentation). Also, the most general results of mechanical and chemical models are consistent. For instance, the effect of domain size on the pattern generated by reaction-diffusion and mechanical models are generally equivalent (see below).

(b) 'Morphostatic' versus 'morphodynamic' models

However, 'pure' reaction-diffusion models should only be applied to systems which do not involve significant cell/tissue movements and growth. They may be adequate for most of the pigmentation patterns of mammals and birds where cells do not migrate, but may not if migration follows differentiation (as pigment pattern formation in amphibians or carcinoma formation, see Olsson & Löftberg, 1993). To some extent, the study of early gene expression patterns (like *Drosophila* 'segmentation genes') seems to be adequately accounted by reaction-diffusion-like models (Salazar-Ciudad, Solé & Newman, 2001; Salazar-Ciudad, Newman & Solé, 2001).

An intermediate position between chemical pre-pattern models and mechanical models can be endorsed if one simulates a kind of feedback between morphogens concentration and other cell behaviors (mitosis, apoptosis, migration, adhesion, etc). One way to do this is to allow tissue geometry (which is at the end dependent on differential cell growth) to influence somehow the profile of morphogens concentration. For example, Salazar-Ciudad & Jernvall (2002) simulate tooth formation by assuming that genes networks (similar to reaction-diffusion) modulate cell growth, so that tooth morphology is changing while morphogen concentrations are not yet at equilibrium. In return, tooth shape modifies the diffusion of morphogens, and so on. Such models have been called 'morphodynamic' since at any step the developing structure depends on its previous shape and not only on morphogens concentration (Salazar-Ciudad, Jernvall & Newman, 2003). In contrast, 'morphostatic' models assume that cell-cell signaling proceeds before and independently of cell behaviors. In other words, cell-cell signaling is not understood as one among several others cell behaviors. In that way, cell behaviors such as mitosis, apoptosis, migration or adhesion are subordinated to cell-cell signaling (and genetics at the end).

'Morphostatic models' (positional information, 'pure' reaction-diffusion) should not be misleading if used to model morphology directly, letting one think that morphogenesis *ss* is just an epi-phenomenon of cell-cell signaling or chemical pre-patterns. Goodwin (1988, p. 636) captures the core of the issue by saying that "*Turing's achievement was remarkable, but it does not provide the solution to the problem of*

morphogenesis. The reason is that a spatial pattern in the concentration of metabolites within a developing organism does not itself explain the actual geometry of say, the tentacles on a hydroid, the leaves on a plant, or the limbs of an amphibian. Morphogenesis, as the name implies, is the generation of structures of specific shape, whereas spatial patterns of chemical concentration arise within some pre-defined geometry. In order to get morphology, work has to be done in deforming cells or cell sheets into specific shapes, and growth must be localized to generate specific structures".

This is not meant to say that 'morphostatic models' are irrelevant to the understanding of development. For instance, Waddington (1956, p. 416) suspected "*that a developing pattern will be influenced by the shape of the area or mass in which it is forming and we shall find examples which demonstrate that this is the case"*. Confirming this expectation, several models investigated the relationships between pattern formation mechanisms and size/growth (e.g. Wilby & Ede, 1975; Murray, 1989; Meinhardt, 1995; Kondo & Asai, 1995; Varea, Aragón and Barrio, 1997; Dillon & Othmer, 1999; Crampin, Gaffney & Maini, 1999; Newman & Müller, 2005; Miura *et al.,* 2006), revealing that the resulting patterns were dependent on domain size and geometry (domain effects). One well known example is provided by vertebrate limb development where the number of emerging cartilaginous elements has been suggested to depend on the ratios of length to width (and to depth in three-dimension) of the paddle-shaped limb bud (Newman & Frisch, 1979; Hentschel *et al.*, 2004, Fig. 1b). Domain effects could underlie the increase in the number of cartilaginous elements in the proximo-distal direction of the vertebrate limb. As the digit plate is wide and flattened, it can accommodate a chemical concentration profile that exhibit a higher number of peaks. Interestingly, Hentchel *et al.* (2004) note that the width of the chick limb bud remains nearly constant at the time of cell condensations. But the proximo-distal length of the active zone (from where cells are proliferating) becomes shorter over the course of limb development. Incorporating this phenomenon in their model, Hentschel *et al.* (2004) suggested that more elements would be produced distally, even if the width of the limb bud is fixed.

Similarly, the reaction-diffusion model by Miura et *al.* (2006) is able to account for the phenotype of the mutant mouse *Doublefoot* (*Dbf*): six to eight supernumerary digits with no clear identity are formed (Fig. 1b). These authors noted that morphologically the autopod (digit plate) of these mutants is 2 to 2.5 times the width of the wrist, in contrast to 1.5 times in the wild-type autopod. These authors go on to show that similar 'domain effects' could underlie the formation of some 'thin-digits' in the *Dbf* mutant: digits can be extremely thin in the proximal region of the metacarpus/ metatarsus (arrows) and discontinuously become 'normal thickness' more distally (Fig. 1b, star). If duplicated digits can be quasi-trivially explained by positional information when one assumes a mirror-image of morphogen concentration (Fig. 1a), the 'thin-digit' phenotype seems more difficult to account for using positional information.

More generally, similar domain effects are expected from mechanical or mechano-chemical models. Contrary to what may be implicitly stated in most developmental biology textbooks, growth factors are not sufficient to make cells proliferate, since cells will not grow in response to saturating growth factors if they are not bound to the extracellular matrix or if they are mechanically compressed (see

references in Ingber, 2002). In the same way, morphogens alone explain neither the patterns of cell differentiation nor their possible neoplastic transformation, nor their death. Indeed, changing the 'epigenetic' context of cell growth (e.g. tissue geometry, cell culture) may typically lead to cell dedifferentiation, neoplastic transformation or their renormalization both *in vitro* and *in vivo*. For example, Ingber (2002, p. 553) reviews many experiments from his and other labs that "*demonstrate that cell distortion per se governs whether individual cells will grow, differentiate or die when stimulated by soluble mitogens as well as the direction in which they move*". Thus, cells can be switched between growth, differentiation, apoptosis or migration *in vitro* when raised on variously sized micropatterned substrates which cause cells to be more or less distorted and probably more or less sensitive to saturating growth factors (Ingber, 2002).The local tensile stress experienced by cells is thus expected to generate local proliferation sites even if cells are submitted to a saturating growth factor concentration. For instance, a developing mammary gland tissue is then expected to change its shape with growth rather than growing homogeneously.

Most of the models of reaction-diffusion proposed so far did not explicitly assumed dynamic feedback between chemistry and growth in the 'morphodynamic' sense. As the chemical pre-patterns are taken to be faster than growth, the chemical concentrations reach an equilibrium state independently of the growth process they may trigger (or may be triggered by other means). It is actually extremely difficult to simulate such dynamic feedbacks with the help of continuous modeling approaches, upon which the vast majority of models of morphogenesis rely (but see Dillon & Othmer, 1999 for a model considering reaction-diffusion on growing domains). Cell-cell interaction models (cellular automaton models and related approaches) can compensate for this inconvenience and find here an interesting application.

(c) Discrete models

Such discrete approaches have not been really discussed so far because they are actually unclassifiable according to my scheme. It seems that this branch of 'theoretical developmental biology' generally does not decouple pattern formation and morphogenesis *ss*, much in the way advocated by Murray (1989). However, some ambiguity comes from the fact that some authors use the term morphogenesis in a wide sense necessarily encompassing pattern formation, much like 'Waddington's individuation' (e.g. Marée, 2000), whereas for others it is pattern formation which is encompassing morphogenesis *ss* (e.g. Deutsch and Dormann, 2005). For some other authors, the two terms are used in alternation or in conjunction with a 'and' locution probably reflecting Waddington's dichotomy between 'regionalization' and 'morphogenesis *ss*'. However, in these cases, pattern formation and morphogenesis *ss* are not necessarily viewed as resulting from chemical *versus* mechanical processes, as it is much often implied in 'experimental developmental biology'.

Cellular automaton models have been used as discrete approximations of reaction-diffusion models (Cocho, Perezpascual & Rius, 1987; Plahte, 2001; Deutsch & Dormann, 2005), as a general scheme to reproduce cellular oscillators (Jaeger & Goodwin, 2001), as tools to investigate more realistic cell-cell signaling biochemical pathways (Collier *et al.,* 1996), or as cell-cell interaction models favoring comparison with *in vitro* experiments (e.g. Kiskowski *et al.,* 2004, Deutsch & Dormann, 2005). Some extensions of

discrete models allow the simulation of more explicit mechanical forces by taking into account cell geometry and cytoskeleton dynamics, and are known as Pott models (Glazier & Graner, 1993; Chaturvedi *et al.,* 2003; Izaguirre *et al.,* 2004). So far, such models were mainly 2D approximations, but 3D models, with or without coupling with continuous approaches, become more widespread (Pallson, 2001; Cickovski *et al.,* 2005; Poplawski *et al.,* 2007).

(4) Pattern formation and morphogenesis as two independent questions?

In conclusion, 'the experimental developmental biologists' patterning and the 'theoretical developmental biologists' pattern formation both endorse a preponderant role of chemicals and cell-cell signaling in driving spatial organization and view static gradients (with respect to other cell behaviors) as a causal explanation of the spatio-temporal heterogeneity of developmental processes (see Tables 1 and 2; Fig. 1a, b). However, the latter view emphasizes a dynamic picture of gradient formation and provides a heuristic explanation of their emergence. As these gradients are not necessarily monotonic, they seem closer to the early 20th century morphogenetic field concept, invoking a molecular pre-pattern (Turing, 1952; Kauffman, 1993; Jaeger & Reinitz, 2006).

On the other hand, no sharp distinction can be drawn between the 'theoretical developmental biologists' pattern formation and the 'biophysicists' morphogenesis (*sl*), insofar as no chemical pre-pattern is assumed *a priori* and some kind of feedback on gradient formation is potentially allowed ('morphodynamic' view). Cell-cell interaction models are largely unclassifiable according to this scheme: they can focus on chemistry or mechanics or both, generally in an abstract way, but not necessarily; they can assume pre-patterns or not, treat morphogenesis *ss* as a slave process, similarly to what has been reproached to some 'pure' reaction-diffusion models (especially when they have been interpreted literally). Cell-cell interaction models can also view morphogenesis *ss* as an active process working in conjunction with cell-cell signaling, thus coming in close similarity with earlier proposed continuous mechano-chemical models (Oster *et al.,* 1988; Murray, 1989).

We should be careful about using the plastic term pattern formation (when it implies only cell-cell signaling) as a synonym for morphogenesis *ss*. This may lead to unfortunate short-comings as exemplified by the tendencies of some authors to reduce any morphology to morphogenetic signals or gene expression patterns. One consequence of equating pattern to morphology is that it conveys the illusion that no physical forces are necessary to explain the generation of form, that is to say that development can be treated as a black box hidden behind genetics studies. Although claims relying on strong 'genetic determinism' have quite regressed in recent years, it is still quite frequent that the question of morphogenesis *ss* be relegated to different time or spatial scales, so that it can be more easily put aside (*a priori* or pragmatically). A consequence is that *"[d]evelopmental biology became the study of differential gene expression rather than the attempt to identify the rules underlying morphologic form"* (Gilbert & Sarkar, 2000, p. 6).

In addition we must take caution about the somewhat 'unnatural' assumption of considering pattern formation (when it means spatial arrangement of structures or motifs) and morphogenesis (3D shape) as separate issues invoking different

mechanisms (cell-cell signaling *versus* other cell behaviors) that could be tackled independently of each other. Several authors already argued for such a limitation and the distort picture it could provide (e.g. Harris, Stopak & Warner, 1984; Murray, 1989; Kauffman, 1993; Salazar-Ciudad & Jernvall, 2004). As recalled above, pigmentation patterns does not necessarily imply that only cell-cell signaling is involved. In return, in most cases, morphogenesis can not be adequately understood as a two step process where cell-cell signaling can be separated from other cell behaviors. For instance, it has been recently demonstrated (Dudley, Ros & Tabin, 2002; Sato *et al.*, 2007) that extensive proliferation and cell mixing in the distal parts of the limb were occurring at early stages of chicken limb development (stages 17-20, Hamburger & Hamilton, 1951). As such, it should be questioned whether limb development provides such a good example of pattern formation[5]. Interestingly, even authors deeply endorsed to mechanics and mechanotransduction still tend to support the view that early limb development is mainly under the control of cell-cell signaling (e.g. Newman & Müller, 2005; Henderson *et al.*, 2007), much in the way proposed by Wolpert & Hornbruch (1990). However, at least from a theoretical point of view, this position seems a bit questionable. If diverse mechanical forces can be attributed an important role in determining the number and location of mesenchymal condensations and the final shape of skeletal elements, as many lines of evidence point out, how could these forces be absent from early stages? It is true that the mechanics of early limb development have not been the focus of much research. But the mechanical effects of the apical ectodermal ridge (AER, Fig. 1a) possibly influencing the shape and growth of the limb mesenchyme from the early stages can not be overlooked *a priori*.

The different views pictured above seem difficult to integrate. This is well illustrated by the manner each of these three views relates to the fashionable concept of self-organization, which is still waiting for a formal undisputable definition in other respects.

IV. Self-organization: a buzzword?

(1) What does self-organization mean?

Many definitions of self-organization have been proposed depending upon the historical context and the level of organization under concern. This term owes its origin to the problematic raised by Emmanuel Kant (1724-1804) concerning the relationships between 'parts' and 'whole'. Kant, one of the fathers of structuralism, emphasized that the 'whole' cannot be understood as only the sum of its 'parts' (e.g. Van de Vijver, Van Speybroeck & Vandevyvere, 2003). On the one hand, Kant opposed himself to the atomization of 'parts' and on the other hand to the superiority of the 'whole' to propose an intermediary relational perspective between 'parts' and 'whole'. As explained by Webster & Goodwin (1982, p.18), "*Kant had argued that what specifically distinguished organisms from mechanical devices, like docks, was that they were self-reproducing and, therefore, self-organizing wholes. This means that, whereas in a mechanical device the 'parts' only exist for each other, in the sense of being conditions of each others function in relation to a common end, so that a machine is a functional whole or unity, in an organism the 'parts' not*

5 By the way, the two 'competing versions' of the positional information model, namely the 'early specification model' and the 'progress zone model' suffer from some of the shortcomings of the original model of Wolpert. They won't be discussed in the remainder of this paper, because the arguments developed below would equally apply to them (see below).

only exist for each other but also ***by means*** *of each other in the sense of somehow producing each other, so that an organism is a functional and a structural unity*". Perhaps, a more intuitive definition is provided by Waddington (1947, p.145-146): "*a new level of organization cannot be accounted for in terms of the properties of its elementary units as they behave in isolation, but is accounted for if we add to these certain other properties which the units only exhibit when in combination with one another…We feel no conviction that, for instance, the behaviour of a mass of tissue must be explicable in terms of the properties of its isolated cells. Instead we hope that investigation of the tissue will reveal new data about the mutual interactions of cells when aggregated in a mass*". In more recent terms, the self-organization concept is associated with the idea of decentralization, and is used to bring about the notion that there is no dichotomy between the organizer and the organized (e.g. Webster & Goodwin, 1982). It points out that a controller or a discrete polarizing region is unnecessary to account for the emergence of organization in (developmental) systems if one assumes instead a feedback between the 'parts' and the 'whole'. In other words (Alvarez de Lorenzana, 1998, p. 70), "*[t]he system constructs its own-order in the process of organizing itself*". This organization is achieved according to the rules of interaction that describe the system's behavior upon given specific constraints and initial boundary conditions. Research on cancer provides a concrete example of this argument. According to the 'somatic mutation theory'-the dominant paradigm under which much of the research on cancer is driven-cancer formation is understood as resulting from an unrestricted cell proliferation due to mutations. This definition of cancer naturally leads to the investigation of the intra-cellular properties of neoplastic cells in order to understand what makes them different from other cells, especially with respect to the molecular control of their life cycle. However, some authors put into question this definition of cancer on philosophical, experimental and theoretical grounds (Ingber, 2002; Aranda-Anzalo, 2002; Soto & Sonnenschein, 2005; Rubin, 2006). For instance, Ingber (2002, p. 547) state that "*[t]he reality is that cancer is not just a disease of the cell…we must go beyond current reductionist approaches that focus on analysis of the abnormal properties of individual tumor cells*". The alternative definition of cancer is that it is a disease of tissue organization affecting cell-cell interactions and linked to the release of generic constraints (such as cell attachment to the extracellular matrix). This view of cancer implies that the behavior of a tumor cannot be expected to be explainable in terms of the individual characteristics of the tumor cells. It argues for a higher level approach of cancer formation, an approach where the behavior of the 'whole' cannot be understood in terms of the properties of the isolated 'parts'. This constitutes what I will call below the first definition of self-organization.

Other definitions of self-organization have been proposed on more pragmatic grounds in particular research fields. For example, self-organization is often defined with regards to non-linear dynamics and complex systems, especially if the system is able to achieve emergent (new) qualitative characteristics through bifurcations (second definition). However, if a system exhibits a very stable homogeneous state, or if the system state is thought to change more quantitatively, does it means that it is not self-organized in the Kantian sense (first definition)? For instance, Horder (1993, p. 141) clumsily condemned D'Arcy Thompson's grids

of transformation, because they fail to *"explain changes in number or differentiation of body parts"*. Similarly, modelers using discrete cellular automaton approaches are prone to define self-organization as 'local rules of interactions' (e.g. Deutsch & Dormann, 2005, third definition), but what if the interactions cannot be reduced to local rules? The problem is that the latter two restricted definitions (2 and 3) of self-organization depend on the scale of observation and necessarily lead to exclude the higher level approaches of biological organization (see below). It seems that the 'self-organization' concept necessitates a certain degree of scale independency since the same phenomenon can be allegedly described as self-organized or not (in the restricted senses 2 and 3) depending on the level of investigation.

For other researchers, self-organization takes the restrictive meaning of emergence of 'heterogeneities' from initially 'random' or 'homogeneous' initial conditions (fourth definition). Of course, whether conditions are described as homogeneous or heterogeneous depends on the scale of observation. Usually, this restricted definition of self-organization is related to the most paradigmatic 'models of self-organization' that assume no initial spatial differentiation in chemical concentrations or cells' types, and thus homogeneous conditions in theory (e.g. see Belousov-Zhabotinskii reaction, Turing patterns). For this reason, self-organization is from time to time equated with such parsimonious assumptions. For instance, Maini (2003, p. 9656-9657, my emphasis) writes that *"[t]here is inherent in the oocytes positional information that must guide pattern, but cells that are completely dissociated and* ***randomly mixed*** *can recombine to form periodic spatial structures. This leads to the intriguing possibility that* ***at least some aspects*** *of spatio-temporal patterning in the embryo arise from the process of self-organization… although self-organization may provide an elegant means of producing patterns* ***de novo****, pattern formation in biology may sometimes depend more on sequential elaboration of initially simple asymmetries"*. Such a statement seems to be rooted in a confusion assimilating the existence of pre-existing patterns (inhomogeneous or asymmetric initial conditions) to pre-patterns (that directly foretell the outcome). But assuming self-organization in the Kantian sense (first definition, no dichotomy between both organized and organizer) does not imply that no pre-existing pattern can influence the system. Indeed, any pre-existing pattern defines initial and boundary conditions. For example, asymmetries in the extracellular matrix, gravitational field, or previously established molecular gradients are understood as model parameters. Then, the system 'organizes itself' (*de novo*) from these asymmetric initial conditions. After all, the distribution of residual stresses in a tissue at one instant can be viewed as an anisotropy pre-existing to the next step of growth. But should we really consider it as a pre-pattern and deny self-organization? Yet, Maini (2003, p.9657) concludes, *"[o]f course, biology is much more complicated than chemistry, and so, whereas in the latter we now have several well identified and studied examples of self-organization, in the former we do not yet have the molecular detail at hand to support or refute the self-organization hypothesis"*. But what is to refute in the cases discussed by Maini is the simple Turing model, not self-organization. In the Kantian sense, self-organization becomes a buzzword for it can be understood as a characteristic of 'open systems', to which developmental systems are an obvious example. The tendency of some authors to oppose positional information and self-organization can

have some important consequences, the most basic one being that it reinforces the idea that instructive metaphors implying controllers are necessary to describe and explain development (see Nijhout, 1990 for a discussion).

It is clear that the tenants of the 'positional information' concept do not consider self-organization (in any of the outlined definitions above) as a fundamental characteristic of developmental systems. For example, in the textbook of Wolpert and co-authors (Wolpert *et al.,* 2006), the only entry for self-organization is to be found page 354. This paragraph is entitled: "*Self-organization may be involved in the development of the limb bud*". One reads: "*the fact that well-formed cartilaginous elements can develop at all in the absence of a discrete polarizing region shows that the bud has a considerable capacity for self-organization"*. Since cells had been mixed and randomized, it is the fourth mentioned definition of self-organization (emergence of a heterogeneous state from homogeneous initial conditions) that is implicitly endorsed by these researchers. However, the explanation that follows makes use of instructive metaphors to account for the fact that 'self-organization' (definition 4) only generates a basic pattern of equivalent cartilaginous elements that need to be specified by positional information in order to achieve their final identity (e.g. humerus, digits). Then, this restrictive definition of self-organization let live the idea that biological form can only be understood thanks to the recourse to a historically given 'central directing agency' or instructive metaphors.

The 'theoretical developmental biologists' generally embrace one of the restricted definitions of self-organization (definitions 2, 3 or 4) that originated in the 40's-50s with the development of the theory of Information and the Cybernetics.

On the other hand, 'biophysicists' generally do not speak about self-organization, except in the works that fit the restricted definitions 2, 3 and 4 (e.g. mechanical instabilities). But in a general manner, the word self-organization is rarely to be found in these studies. The problem is not that these definitions are intrinsically flawed. They work well in restricted research fields. But taken too literally, or used to compare methods in different research fields, these restricted definitions can lead to marginalize other approaches such as those that describe the self-organized phenomena at a higher level of organization.

(2) Why is self-organization uncomfortable to deal with?

One difficulty in accepting that development is self-organized comes from the assumption according to which self-organization is not robust enough. For instance, reaction-diffusion models have been criticized for not being robust enough to account for the stability of the morphogenesis (e.g. Bard & Lauder, 1974). In the same way, Wolpert (1996, p. 363) said that "*it is not easy to believe that if morphogen gradients do indeed control pattern formation, they should rely on simple diffusion through the extracellular space. Such a mechanism seems just too unreliable and a more sophisticated mechanism would surely have been 'invented"*". Actually, some authors highlighted plausible ways for increasing robustness in reaction-diffusion models (e.g. Crampin, Gaffney & Maini, 1999) and more widely in gradients' models (e.g. Eldar, Shilo & Barkai, 2004). Even though we know little about the mechanisms underlying robustness in development, it seems that this characteristic of development relies more upon the distribution of control

over the entire system (feedback) rather than upon particular material entities ('controllers' such as genes). It means that self-organization itself could be viewed as the origin of robustness.

Another difficulty in conceiving that there is no such thing as non-self-organized development can come from the fact that most of the time self-organization is counter-intuitive. Viewing development as a self-organized process also challenges the linear thinking at the core of molecular biology. Moreover, complexity poses epistemological problems that need to be addressed, especially circular causality and multi-scale causality (feedbacks among and across levels of organization). In addition, understanding complexity requires a kind of logical complexity, which is known as dialectics (Levins & Lewontin, 1985). Whether the sciences of complexity could benefit from the use of dialectics still needs some investigation. This requires the foundations of dialectics to be discussed and reappraised by epistemologists and historians of science.

But as Nijhout (1990, p. 443) noted: *"[t]he main difficulty in accepting development as a self-organizing process is that we do not have a simple description of heritability and self-replication for such a system"*. The difficulties in understanding heredity have been temporally bracketed with the Weismannist dichotomy that viewed the nucleus (and only the nucleus) as the support of heredity. But heredity is not a simple concept anymore if one does not assume (at least implicitly) a causal priority of genes.

(3) Mathematical models *versus* metaphorical language

In formulating the positional information concept, Wolpert intended to redefine the loosely defined 'morphogenetic field' concept and wanted to draw attention to pattern formation, a question which was not really in the mainstream interests of the time (Wolpert, 1969). And he succeeded in doing so as this concept undoubtedly triggered and guided many experiments. In some important way, Wolpert's scheme also contributed to raise the developmental biologists' interest in general principles (it is worth to note that molecular genetics have long refuted the universality of positional information). However, the positional information concept seems to have outlived these advantages and several authors have warned against the distort picture that such a simple linear concept can convey, potentially obscuring some detailed investigations of the regulatory processes (Gilbert, Opitz & Raff, 1996; Salazar-Ciudad & Jernvall, 2004; Salazar-Ciudad, 2006b; Jaeger & Reinitz, 2006).

As criticized by Webster & Goodwin (1982, p.36), *"Wolpert's scheme is completely Weismannist in reliance on the historically given 'central directing agency' as the determinant of form, and morphological diversity is inevitably, therefore, seen as irreducible. There can be no general 'laws of form' in such a theory since the only universal, positional information, imposes no constraint upon the form which is generated other than that it be spatially extended"*. Wolpert proposes a duality between a universal positional information principle and its specific interpretation by the cells. Molecular gradients may well be universal as we can be sure that some spatial heterogeneity of gene products will affect morphogenesis in some way. However, Wolpert does not provide any operational explanations of the way cells are to interpret pre-formed gradients, so that his proposal is unconstrained. The location of the thresholds along (monotonic) gradients involves *ad hoc* justifications (*a priori* knowledge

of the morphological outcome to be explained is required). The lack of conditional hypotheses is unfortunately a common (though not obligatory) pitfall of models proposed in a narrative form (it is much less frequent with mathematically framed models).

These arguments are hardly new. As put by Alberch (1993, p. 458-459) some fifteen years ago, *"Wolpert, ..., in his enthusiasm for the new data emerging from molecular biology understates a problem that is central, not only to developmental biology, but to the study of any system in which billions of components interact to generate an orderly pattern: 'the problem of complexity'...The difference between the instructions 'to make a leg' vs. 'to make an arm' is probably not qualitative at the genetic level but quantitative. Variations in the context ('boundary or initial conditions') can generate very different outcomes even if the rules of interaction remain invariant. How to balance this liability with the invariance that characterizes development remains an unsolved problem, and I doubt it can be tackled strictly from a molecular and reductionist perspective. One needs to invent or rescue from the mathematical literature, new tools to study the behaviour of complex dynamical systems".* Thus, Alberch's advice has been recently put into practice since insightful data-driven mathematical approaches have been developed and applied to the study of regulatory processes in a way that do not understate their dynamical properties (e.g. Jaeger *et al.,* 2004). As Jaeger & Reinitz (2006, p. 1109) conclude, *"[m]athematical models allow us to keep track of the many simultaneous regulatory processes and feedbacks occurring in a field, and to cope with the complexity of intact, wild-type developmental systems. It is difficult to imagine how we could have unraveled the nested regulatory feedback loops that cause dynamic shifts in gap domain boundaries without the help of computational modelling... This is an important methodological advance, since it enables us to link the dynamical properties of an intact morphogenetic field to specific regulatory mechanisms in a way that is difficult to achieve by traditional experimental means".*

Computer simulations are more than necessary for us to deal with the non-linearity of development. The self-organizing principles of development do not have to be very elaborate to rapidly and easily counter our intuitions. 'Narrative models' and their associated metaphors do not allow us to deal with the complexity of biological organization, especially as a quantity of details is available[6]. Even though metaphorical language has a strong undeniable heuristic power, "*the price of a metaphor is eternal vigilance*" as Lewontin (2000b) likes to repeatedly remind. Equations remain the most efficient way to account for the covariation between variables and to tackle the dependence of a system upon its context (see references in section VI for examples).

Mathematical models usually allow the easier prediction of the outcome and the testing of various conditions, and they often provide a different interpretative view of the problem. For example, with the help of their model, Jaeger and Reinitz (2006, p. 1109) convincingly demonstrate "*how one of the most important examples of a developmental process thought to be governed by strictly instructive and hierarchical developmental signals in fact relies on regulative feedback. This suggests that static metaphors—such as that of an embryonic coordinate system—are of limited use and that the fundamentally dynamic*

6 By the way, note that mathematical models are far from being free from metaphors too.

nature of all developmental phenomena should be reflected in the concepts, and methods, used for the study of embryogenesis". Yet, Gilbert and Sarkar (2000, p. 8) quite sadly note that "*one would have to look very carefully in any of the major developmental biology journals to find a differential equation or any other type of quantitative analysis*". Hopefully things are changing (e.g. Jaeger *et al.,* 2004). Similar approaches extending beyond regulatory networks will probably become more and more tractable and made more widely visible to scientists working in distinct fields.

(4) External static view *versus* dynamic internal view

Wolpert (1969, p. 18) cites Waddington who "*has pointed out that the term 'field' should only be used to refer to the character of the process occurring in a region or district and should not be used simply to refer to the spatial location of, for example, a presumptive region*". Wolpert thought that his redefinition of Waddington's field concept in terms of positional information was fulfilling Waddington's criteria, mainly because positional information has the ability to account for some observed regulative properties of fields. But in defining fields by means of an external fix coordinate system, Wolpert is taking an external observer point of view on the system. As argued by Jaeger and Reinitz (2006, p. 1109) *"Wolpert's fields have lost important features of the original field concept [Waddington's definition]. The latter relies on the complex, interacting processes occurring within the field to define its regulatory and developmental capabilities. In contrast, Wolpert's field concept considers processes occurring within the field as irrelevant for its definition. Instead, it relies on the idea of a common coordinate system, and is therefore defined by purely spatial rather than regulatory relationships".* Perhaps paradoxically, one reason for Wolpert's scheme ability to account for some regulative properties of fields stems mainly from the kind of generic behaviour that the positional information concept allows. Although Wolpert and his followers interpret positional information with metaphors related to 'instructions' and 'specificity', this concept seems to owe its experimental validity to the great flexibility permitted by the decoupling of positional value assessment from its interpretation by the cells.

The crucial difference between Waddington's 'morphogenetic field' (1947, p. 144) *"regarded as the product of the interaction of its parts"* and Wolpert's 'morphogenetic field' redefined as purely external spatial relationships apparently relies on a shift of the observer position relative to the system from an internal one (thus necessarily dynamic) to an external one. As such, the external spatial coordinate system implied by the positional information concept may be thought of as a being in the mind of the beholder. Artificially imposing an external static coordinate system on a dynamic system may be misleading if the positional information concept is taken too literally, for it naturally leads to searching how cells acquire positional values, how they detect thresholds, how sharp boundaries are created, etc. It may divert attention from the necessity of non-linear quantitative studies. Positional information is probably the simplest 'principle' of development, and as such, it can not avoid reminding us Whitehead's motto (1919): *"seek simplicity then distrust it"*.

V. 'Generative structuralism': a way toward a theory of form?

Two apparently opposed properties of organisms are robustness and variation. Robustness refers to developmental systems' tendency to reach similar outcomes in spite of perturbations (be they genetic mutations, environmental changes, experimental manipulations and even changes in the rules of interaction).

'Generative structuralism' can help to provide a picture where robustness and variation can simultaneously be accounted for. 'Generative structuralists' define (morphogenetic) events as transitions from one structure to another. The knowledge of generative rules and initial and boundary conditions provide an explanation of the (morphogenetic) event. 'Generative structuralism' addresses the question of the necessary conditions of transformation of structures, their variation and their potentialities for evolution (see Piaget, 1972 for an extensive discussion).

Although structuralism has a long history in science, it regained interests toward the last century in different scientific domains related to biology or inspired by problems coming from biology. We can trace back four roots at least that simultaneously revived these concerns between the 40's and the 70's by reintroducing in the debates the concept of 'system' and its conjoint concepts of complexity and organization: cybernetics and artificial life, co-emerging with the questions of self-replication; general system theory including a theory of open systems; biomechanics, concerned with the application of continuum mechanics to the study of hard tissues growth and repair; and the fields of psychology, sociology and behavior sciences, promoting the development of 'constructivist theories'.

Such approaches require that, in first instance, variation is abstracted in order to highlight some common characteristics underlying apparently disparate phenomena (topological relations). Then, a system can be constructed to describe and to analyze the interactions between the system components and the conditions that allow organization to occur (emergence), thus accounting for the generation, maintenance or transformation of structures.

In the tradition of the 'Entwicklungsmechanik' of Wilhelm Roux, the 'generative structuralists' (or organicists, see below) seeking for 'the universal laws of biological order' (a theory of transformation of morphological structures) repeatedly laid strong emphasis on the most neglected explanation of morphogenesis *sl*: mechanics (e.g. see Beloussov & Grabovsky, 2006). Towards the end of the 19th century, the embryologist/physiologist Wilhelm His (1888, p. 293) was already stating that *"[e]mbryology and morphology cannot proceed independently of all reference to the general laws of matter, - to the laws of physics and of mechanics. This proposition would, perhaps, seem indisputable to every natural philosopher; but, in morphological schools, there are very few who are disposed to adopt it with all its consequences"*. Since nearly four decades, a revival in biomechanics interests has produced seminal insights that confirm both experimentally and theoretically the importance of mechanics to developmental biology, cancer research and tissue engineering (see references in section VI). But first, I need to highlight what the main principles of 'generative structuralism' are. Then, I will discuss some of the usual critics against the structuralist framework. I will argue that 'generative structuralism' and its often associated dialectical approach constitute an adequate framework to study the complexity of development and evolution.

(1) On formation and transformation of structures

(a) The identification of topological regularities among biological forms: the concept of 'Types' of rational morphologists

It has been extensively argued that 19th century pre-Darwinian rational morphologists (and their supposedly modern structuralist counterparts as well) were not concerned with variation because of their *a priori* idealist-essentialist philosophy (e.g. Mayr, 1963). Because of these metaphysical concerns, it was also widely claimed that rational morphologists were assuming 'species fixism', and thus were depicted as antievolutionists, in the same line than Natural Theologians. It is not the place here to discuss the essentialist critics that rational morphologists have been blamed of from the 60's to nowadays in several historiographies, but it is pointed out that some of these critics (and surprising shortcomings) have been reinvestigated in depth by Amundson (1998; 2005). Here, I follow the revised historiography of this author to provide a crude sketch of the 'Types' problem and I apologize for extremely oversimplifying it.

Rational morphologists were primarily concerned with the classification of organisms according to the empirical regularities they observed in spite of the diversity of adult and embryonic forms. It turned out that these regularities were invariant structural relationships, and it allowed the building of abstract 'Types' (or Baupläne) which represented the set of topological relationships common to a variety of forms (although there is plenty of species that do not fit into the definition of the Bauplan of their own phylogenetic group).

It has been argued that assuming species fixism at that time was scientifically progressive (Amundson, 2005). In retrospect, it is in fact difficult to imagine that the phylogenetic classification of life (Natural System) could have been built at all under the 18th century assumption that every 'transmutations' were possible. However, regarding their metaphysical concerns, the rational morphologists are far from constituting a homogeneous group and virtually nothing in their writings points to essentialism and idealism as a ground for species fixism (Amundson, 2005).

Moreover, their focus on 'Types' sounds more like a 'rational' choice in the attempt of classifying and investigating the ordered diversity of life rather than a commitment stating that variation was an unimportant phenomenon. As put by Webster & Goodwin (1982, p. 19), "*[Rational morphologists] were primarily, though not exclusively, concerned with 'Being' or 'Order', with the universals hidden in diversity and the permanence behind change-which does not exclude consideration of change. The empirical 'laws' they were concerned to discover were formal laws which would enable the multiplicity of 'given' forms to be reduced to, that is, to be described in terms of, a small number of general relational statements*".

Their work revealed 'hidden similarities' transcending the variation in shape and function (homology in modern terms) among organisms. These results were further interpreted by Darwin as evidence for the genealogical relationships of species ('common descent with modification'). On one side, this homology concept put into evolutionary perspective by Darwin allowed the assessment and construction of the evolutionary theory; on the other side the structural homology concept allowed the raise of embryology and comparative anatomy, approaches that nowadays are widely defended as indispensable to a complete evolutionary theory. Thus, the contribution

of the 19th century's morphologists has recently been rehabilitated by a number of philosophers and historians (Greene, 1992; Young, 1993; Love, 2003), as well as by biologists that place such contributions at the core of the now called 'evo-devo' research field (Webster & Goodwin, 1982; Hall, 1996; Gilbert, 2003).

In any case, the rational morphologists' silence concerning evolution should not be intended as a strong antievolutionary commitment; at least it does not justify their traditional categorization among the 'special Creationists', as it has unfortunately often been the case in syntheses of evolutionary thought. Once that one sets apart the idealist philosophy (similar to Kantian's view) that some (not all) rational morphologists endorsed, their failure to assert the phylogenetic relationships between organisms can be understood as a perfectly respectable scientific cautionary attitude with respects to the natural causes that they eventually assumed but were unable to name and investigate at that time (Amundson, 2005). "*True enough, the concept of a hierarchy of Baupläne originated with the idealistic morphology of the nineteenth century, but this should not prevent its existence from becoming the object of modern empirical research"* (Rieppel, 1990, p. 307). But whatever the history of 'evo-devo' predecessors, the actual research questions are: "*how does a Bauplan originate in ontogeny, why is it conserved through geologic time, what is its potentiality for change, and how is this constrained?*"(Rieppel, 1990, p. 307).

(b) Dynamic properties of developmental systems: a way toward reconciliation of homeostasis and homeosis

Since the 80's, it has been recognized that the explanation of inherent topological regularities of organism morphologies upon which any phylogenetic constructions are based (homologies, types, Baupläne) are to be found into the dynamical processes which generate them (see Webster & Goodwin, 1982; Goodwin, 1988; Alberch, 1989; Kauffman, 1993). The relative level of conservation of body plans has been proposed to be caused by the generative properties of developmental systems, and not due to the conservation of genetic information (Hall, 1996). These generative properties, stemming from the dynamic interaction of 'parts' and 'whole' set out the constraints and the possibilities for variation of biological forms.

The most basic concept is that biological forms are constrained by the possibilities that the rules of chemistry, physics and geometry allow (Thompson, 1952). In other words, the set of theoretically possible forms is bounded by the way these forms are generated. Historically, the concept of 'developmental constraints' has been used to challenge the 'Modern Synthesis' which denied that development could play any role in determining the direction of evolution. In particular, 'developmental constraints' were used to argue against the view that without selection phenotypic variation would be random (e.g. Alberch, 1980[7]). But, it appears that 'random phenotypic variation' is not an easily definable concept (although it may seem at first sight). Moreover, different meanings have been given to 'random' in the synthesis (Eble, 1999). These complications put apart, it appeared that in practice, the 'Modern Synthesis' assumed that variation was gradual and 'in every direction', a view which has been much debated, at least since the late 70's under the umbrella of 'developmental constraints'.

Sometimes, the term 'constraints' is also used in a somehow different meaning than

7 And see chapter 2.

discussed above. In this usage, one speaks of the mathematical constraints that correspond to the initial and boundary conditions of the system under study. In mathematical models, the behaviour of developmental systems is described, characterized and predicted thanks to the rules of interaction between molecules, proteins, cells and/or tissues under a particular set of constraints (initial and boundary conditions). Without such constraints, the behaviour of a system cannot be predicted. In this view, constraints are given a decisive and 'creative' role.

When critics of the 'Modern Synthesis' pointed out that not every kinds of variation were developmentally possible (first sense), they were arguing that development was limiting the range of possible variation on one side, and that development was creative on the other side (second sense). In this way, the first and second meanings of constraints partially overlap, the first building extensively on the second.

Probably because of this ambiguity in the term 'constraints' (limiting/creative role), it has been advocated that this term would be best replaced by 'developmental bias' (Arthur, 2004).

In the so-called 'sciences of complexity', the generic forms (invariants, typical forms) can be understood as mathematical attractors which represent the possible solutions of non-linear systems given specific initial and boundary conditions (universal generative rules and specific constraints) (Webster & Goodwin, 1982; Alberch, 1989; Emlen *et al.,* 1998; Huang & Ingber, 2000). As noted by Webster & Goodwin (1982), these generic forms or attractors are reminiscent of Bateson's concept of "*positions of organic stability*". In these systems, similar solutions can be reached even if the starting conditions or systems parameters are different. Depending on the set of solutions allowed by the 'laws' of matter under particular constraints, developmental systems may be robust; similar outcomes will be achieved in spite of changing initial conditions (Fig. 2). Its means that the solutions of such systems can be structurally stable (Thom, 1972). The relative (in) sensitive dependence of a system on the initial conditions is intimately related to the topology of theoretically possible solutions in the parameters space. Let a given outcome falls into a particular domain of attraction in this parameters space. If this domain is relatively large, the corresponding outcome will be extremely probable, meaning that it can be viewed as generic. Then, a system characterized by a few large basins of attraction will be relatively insensitive to changes of the input parameters (structurally stable). In figure 2, the basin of attraction representing apoptosis is the deepest and the broadest. It means that it is the most stable outcome of the considered system and can be achieved through many non specific changes in conditions (see Huang & Ingber, 2000 for a discussion).

The trajectories that developmental systems tend to follow have been called 'chreods' by Waddington (1977), a concept emphasizing the canalization of systems and suitable to explain the homeostatic persistence of homologies (robustness or ability for systems to withstand perturbations). This concept of canalization has been given a more formal definition in the'catastrophes theory' of Thom (1972). 'Catastrophes' refer to the discontinuities observed in the outcome when a bifurcation occurs and the system's solution goes to another basin of attraction. For instance, the passage from the basin of attraction marked as representing proliferation to the one representing differentiation in figure 2 would correspond to a bifurcation in the

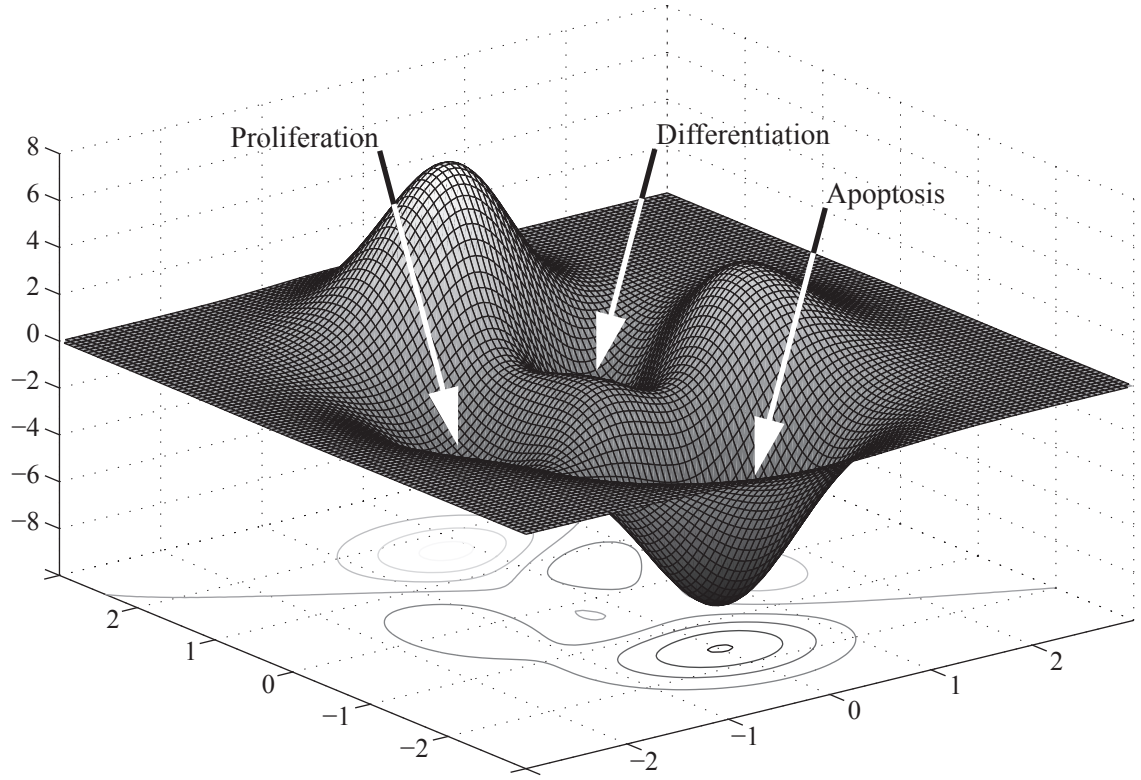


Figure 2: Topology of the solutions (landscape) for the developmental system. The basins of attractions represent distinct cell fates. Every position in the *xy* plane corresponds to a set of initial conditions (2 parameters here for graphical representation). The vertical *z* axis represents the energy required to move the system from one basin of attraction to the other. Systems starting near a valley will tend to stay in this region (structurally stable, see contour plots). Perturbations can make the system cross a hill and fall into another basin of attraction: the system can undergo a bifurcation. Redrawn with modifications from Huang & Ingber (2000).

developmental system.

Such generic properties of organismic systems also appear as a highly possible explanation of the widespread convergence of morphologies (homoplasies) observed in many instances between phylogenetically distantly related organisms. In such a view, homoplasies are understood as resulting from the channeling of developmental systems into 'basins of attraction'. As put by Rieppel (1990, p. 318), "*[c]onvergence, expressed as character incongruences, need not necessarily be explained on functional grounds, but might just as well result from the actualization of shared generative potentials in unrelated taxa"*. A discussion of the alternative views upon the origin of convergence (with concrete examples) can be found in many sources (e.g. Wake, 1991; Hall, 2003; Urdy & Chirat, 2006).

In the same way, generative mechanisms of developmental systems are viewed as the source of origin of novelties (Müller & Newman, 2003), an issue that has been largely ignored by the 'Synthetic Theory'. Some authors investigated *'in silico'* the role of development in influencing morphological evolution (morphospace occupation, developmental constraints). For example, using models of tooth development, Salazar-Ciudad & Jernvall (2004) compare the kind of phenotypic variation produced by different models whose parameters are randomly varied. The relationship between phenotype and genotype can be derived in each case and the relative involvement of various types of developmental mechanisms in the generation of novelties can be estimated.

Now that I have highlighted the basic stance endorsed by 'generative structuralists', I would like to address some of the critics that have been raised.

(2) Critics of structuralism

These critics were directed against structuralists approaches of the 19th century in various historiographies of evolutionary theory. As these structuralist approaches reawaked and challenged the 'Modern Synthesis', these arguments were ready to be directed to the descendants of transcendental morphologists. Of course, most of evo-devo developed after these disputes, so these critics may not concern 'evo-devo' after all (but see Jenner, 2006 for a recent critic of 'typological thinking' in evo-devo). As far as I am aware, these critics have not been discussed in the context of 'evo-devo'. Also, perhaps most of 'evo-devo' developed in the ignorance of these critics. As one defines itself also thanks to critics, I think it could be beneficial for 'evo-devo' to be aware of it, in order not to fall into one extreme or the other. Moreover, the discussion of these critics is important for understanding how 'generative structuralism' proposes to deal with the integration between levels.

(a) Ahistorical?

The 'generative structuralism' advocates have often been criticized for their tendency in considering 'laws' of far more greater importance than historical contingencies, letting some believe that such approaches were intrinsically inadequate to account for the undoubtedly historical nature of evolution.

In the structuralist framework, the historical (contingent) factors are taken into account as starting initial conditions, and structuralism can only explain (describe) the outcome of the system once the historical conditions are met. Structuralism does not come in conflict with the explanation of the origin of these conditions, since it takes them for granted *a priori*. This means that once the conditions are met (and only once they are met), the outcome can be described as inevitable if the system behaves in accordance to a given rule.

But in biology, it seems that different causal ideals exist (e.g. Van der Weele, 1993). Some researchers rather emphasize the crucial (historical, particular or material) factor that conditions the existence of the phenomenon under study, whereas others emphasize the laws describing the system behavior.

Taking the limb studies as an example, note that proteins, such as Shh, are often thought of as a cause of the development of distal parts of the limb and digit identity. As put by Tickle (2003, p. 452), "*The phenotype of Shh$^{-/-}$ mouse embryo limb buds shows that Shh signaling of the polarizing region in the limb bud is critical for proper development of distal parts of the limb. In absence of Shh signaling, structures distal to the elbow/knee are very reduced, with at best a single rudimentary digit developing in the leg… There has been increasing support in recent years for the idea that Shh signaling fulfills two roles in the limb bud -specification of digit number and specification of antero- posterior position and hence digit identity*". If a gene is silenced or mutated, and if the morphogenetic outcome is prevented, a causal role will be given to this gene. But the particular gene which was first thought to be critical can reveal itself unnecessary under some other conditions (e.g. multiple knock out). Although the knowledge of the material entities underlying the hypothesized mechanisms is necessary, it is not at all sufficient.

Many phenomena, such as limb morphogenesis, should also be understood in terms of laws, describing the interaction between particular material constituents.

(b) Essentialist? Idealist?

The structuralist framework, the modern counterpart of typological thinking, has been repeatedly blamed of being essentialist (e.g. Mayr, 1963). But as argued above, the structuralist invariant forms should be understood as the results of homeostatic (dynamical) processes in clear opposition to essentialist conceptions of types (Platonic eternal forms or essences). Homeostasis is observed when self-organizing processes sustain the stability of sets of properties (invariance) in spite of changing conditions.

The idealist conception of 'Types' can be rejected as well. 'Types' can easily be understood as abstract theoretical constructs of sets of properties that are homeostatically conserved. As put succinctly by Levins and Lewontin (1980, p. 69), "*what distinguishes abstractions from ideals is that abstractions are epistemological consequences of the attempt to order and predict real phenomena, while ideals are regarded as ontologically prior to their manifestation in objects"*.

(3) Reducing the problem: scale matters

Structuralism is neither the opposite of historicism nor of functionalism. It is rather the opposite of atomization, which consists in attempting to reduce the explanation of phenomena to their lower hierarchical levels of organization and necessarily imply a linear chain of causation between these levels. Yet, structuralism is not a wholist approach, which would state that everything is connected to everything; a position that would render the search for rules meaningless. Indeed, 'generative structuralism' is close to organicism, in the ways described by Gilbert & Sarkar (2000, p. 1-2): *"The difference between organicism and reductionism is that organicism holds that explanation cannot proceed solely from the properties of fully individuated parts even though all properties of the whole are determined by the properties of the parts. Another way of depicting this disagreement is to picture reductionism as a system where a "bottom-up" approach (e.g., atoms to molecules to organelles to cells to tissues) is sufficient to explain all phenomena. Organicism claims that this is not sufficient and that top-down and bottom-up approaches must both be used to explain phenomena… The properties of any level depend both on the properties of the parts "beneath" them and the properties of the whole into which they are assembled"*. Historically, organicism has been poorly distinguished from vitalism and holism[8] (Gilbert & Sarkar, 2000). Structuralism has rather been blamed for being ahistoric, essentialist or idealist, but has not been strongly connected to holism. Also, organicism provided much of the framework for embryology of 19th to early 20th century, and is perhaps more related to 'mechanistic' embryology than structuralism which is rather more connected to the phylogenetic tradition of morphology. But it is likely that both terms represent nothing more than subtle variants of common tradition (stemming from Kant's work).

Although Gilbert & Sarkar (2000) tend to oppose organicism to reductionism (I would rather say atomism see above), 'generative structuralism' (or organicism) can be understood as a reductionist perspective *a posteriori: "A*

8 (W)Holism privileged the whole and subrogated the parts.

reductive explanation of a system property or behavior shows it to be mechanistically explicable in terms of properties of and interactions among the parts of the system" (Wimsatt, 2006, p. 697).

By comparing the set of theoretically possible transformations to the set of real transformations, structuralism can set up a framework that provides the conditions for the building, maintenance and variation of structures. It highlights that some general properties of systems are necessary to account for the stable and variational properties of structures. Abstraction focuses on the interaction of parts and leads to neglect irrelevant details (the particular material entities underlying the interactions). So for 'generative structuralism' and organicism as well, the main focus is on the interaction among and accross levels. Any level of organization exhibits its own set of rules; rules that can not be analyzed at a lower scale. In return, lower scale phenomena also exhibit characteristics (e.g. variability, stochasticity) that are often not to be found at a higher scale (e.g. Wimsatt, 2006). In this respect, all hierarchical levels of interaction equally deserve careful investigation.

Dialectics can be associated with structuralism in order to reconcile apparent contradictions (and organicism/structuralism have often been viewed as a variant of dialectical materialism, e.g. Waddington, 1947; and see Gilbert & Sarkar, 2000). For instance, structuralism makes use of generality ('laws') and particularity (initial and boundary conditions) in order to explain stability (homeostasis) and variation (any kind of transformation or homeosis) at the same time. These dichotomies (or local/global, qualitative/quantitative, intrinsic/extrinsic, to cite just a few others) can be resolved by their interaction in the framework of dialectical materialism (see Levins & Lewontin, 1985). For instance, Horder (1993, p. 146) notes that *"problems of biological explanation often revolve around a conceptual dichotomy or antithesis, both elements of which very often turn out to be interrelated and equally relevant in the end (e.g. nature/nurture) or the bridging of the domains of evolution and development…Many biological concepts are used in a disjunctive or all-or-none manner… leading to saltationist thinking, which can often be seen to create artifacts which misrepresent the underlying continuities of biological processes"*. The example of 'pattern formation/morphogenesis' or its corollary 'chemistry/physics' discussed in this paper (and mentioned in passing by Horder, 1993) provides an example of the difficulties that arise from this dichotomy. Another example more briefly discussed in this paper is provided by the quadrichotomy between mitosis, apoptosis, differentiation and migration (Fig. 2). These four outcomes represent different qualities of cell fate that can be viewed as representing stable domains of attraction (Huang & Ingber, 2000). The mechano-chemical factors determine which particular state a cell will achieve given initial and boundary conditions.

I will end up by a short review of models in order to prospect about the future of theoretical approaches to morphogenesis. I will point to the different available perspectives on modeling morphogenesis.

VI. Short outlook: the future of modeling morphogenesis *sl*

Modeling of morphogenesis may be tackled from various complementary angles, which allow the reduction of the problem while escaping genetic reductionism. These approaches may focus on low (subcellular) hierarchical levels in

order to analyse the properties of gene networks. But modelers may also focus on the understudied higher levels of organization (tissues) to investigate them heuristically or to integrate new data, emerging mainly from tissue engineering. Alternatively, models mixing different levels of organization may provide some answers to the most disconcerting question of the genotype to phenotype mapping.

Different levels of abstraction may be needed too. At one end of the modeling spectrum, we have highly detailed, specific models relying firmly on quantitative data. These models are aimed at predicting particular behaviors and could be used in some instances as palliatives to experimentation. At the other end of the spectrum, we have abstract models relying on more qualitative observations. These models are aimed at emphasizing the minimal hypotheses that capture some essential generic features of the system's behavior (heuristic approach, null-hypothesis models).

Lower level models typically focus on gene networks and draw a convincing picture of their generic properties (Salazar-Ciudad, Garcia-Fernàndez & Solé, 2000; Salazar-Ciudad, Newman & Solé, 2001). They provide insights into their robustness and evolvability under various modifications such as gene duplications (Aldana *et al.,* 2007). These approaches not only facilitate the integration of experimental data into a comprehensive framework (De Jong, 2002; Jaeger *et al.,* 2004; Reeves *et al.,* 2006, Eungdamrong & Iyengar, 2004), but also allow the classification of genetic networks into groups on the base of the topological relationships between interacting genes, thus probably helping in disregarding many details that, at least in a first approximation, are superfluous. Such approaches could also help in understanding the function of some specific genes based on their role in experimentally well-known gene networks.

Higher level approaches focus on the role of cells in building tissues and emphasize the feedback between developing tissues and their constituent cells (Hentschel *et al.,* 2004). Modeling at this organization level usually requires strong inputs from mechanics to understand the relationships between tissue geometry, tissue-tissue interactions and locally generated forces. Modeling in biophysics can rely on different analogies depending on the phenomenon under study. Tissues composed of motile and adhesive cells can be analogized with liquids (e.g. Steinberg, 1970; Beysens, Forgacs & Glazier, 2000a; Neagu *et al.,* 2005), whereas tissues composed of tightly bounded cells (epithelia) will rather be analogized with elastic materials (e.g. Odell *et al,* 1980; Harris, Stopak & Warner, 1984; Davidson *et al.,* 1999). Tissues can also be considered as viscoelastic materials (e.g. Beysens, Forgacs & Glazier, 2000b), especially when simulating the extra-cellular matrix, or when growth or cell spreading has to be taken into account. On a short time scale, tissues deform much as elastic materials but in the long term, growth induces larger deformations which are rather viscous-like.

Growth/cell migration can result from stresses (tension dependent growth), but growth/cell migration often induces residual stresses in return (e.g. Beloussov, Louchinskaia & Stein, 2000; Cherdantseva & Cherdantsev, 2006). This means that anisotropic growth can in principle destabilize a given tissue geometry even without external loading (Ben-Amar & Goriely, 2005). Moreover, tissue shape can feed back on cells behavior to constrain local growth proliferation patterns according to local stress distribution. Therefore, tissue 3D geometry can be viewed as

an actor of its own morphogenesis (see Nelson *et al.,* 2005 for a discussion of *in vitro* and *in silico* experiments).

Models may have a heuristic value, triggering new experiments and avenues of research, especially when they focus on 'highly' non-linear mechanics and/or do not yet rely upon extensive experimental data (e.g. Odell *et al.,* 1980; Weliky & Oster, 1990; Beysens, Forgacs & Glazier, 2000a; Drasdo & Forgacs, 2000). They can also investigate the range of parameters (e.g. elastic modulii of cells layers and extra-cellular matrix, tissue surface tension) that allow certain phenomena (like the primary invagination of gastrulation or cell sorting) to occur according to the mechanisms supported by observations and experiments (Davidson *et al.,* 1995; Davidson *et al.,* 1999; Foty *et al.,* 1996; Beysens, Forgacs & Glazier, 2000b).

Another promising approach at the cellular level is that of the cellular automata models (or more generally agent-based models) that allow the explicit simulation of growth and cell migration, and its coupling to other cell behaviors, such as cell-cell signaling (Deutsch & Dormann, 2005; Salazar-Ciudad & Jernvall, 2002; Salazar-Ciudad & Jernvall, 2004). Advances in time lapse imaging already provide observations of individual cell trajectories which will soon be used in discrete models, particularly well-suited to the analysis of this type of data. Their coupling with Finite Elements Approaches (FEA), that are already widely used to analyze the structural mechanical properties of tissues, could favor the dynamic modeling of cells behavior in response to the stresses they experience. Gaining more experimental and theoretical insights into the temporal evolution of morphogenesis is a promising road of research.

There is a somewhat growing interest in the development of multiscale models, whose goal is to uncover the disconcerting relationships between microscopic and macroscopic levels of biological organization. Tracqui (2006, p. 722) remarks that *"model driven analysis of the interplay between biochemical and biomechanical cell signaling pathways appears more and more clearly as a necessary step in the field of tissue engineering."* Uncovering the relationships between hierarchical levels of organization is challenging, in part because of the various timescales involved in these phenomena. However, more and more modelers are now able to integrate both experimental and theoretical aspects, so that they can propose a more comparative and quantitative approach (e.g. Salazar-Ciudad & Jernvall, 2002; Cickovski *et al.,* 2005; Tracqui, 2006; Cai, Landman & Hughes, 2007). Tremendous progress has been achieved in the understanding of the connections between microscopic and macroscopic levels thanks to mechanochemical transduction studies (e.g. Ingber, 2002; Nelson *et al.,* 2005). Statistical mechanics, chaos theory and stochastic processes may provide the missing links for understanding the relationships between molecular activities and macroscopic properties of tissues. The interplay between different basic processes (differential adhesion, cell differentiation, cell-cell signaling, cell growth/death) can also be tested with the aid of evolutionary algorithms. They highlight some of the rules necessary to the generation of morphogenetic features (e.g. engulfment) and point to some relatively generic outcomes reappearing during unique evolutionary histories (e.g. Hogeweg, 2000). Up to now, theoretical models of development could be classified into two major mathematical classes (continuous, discrete). But it is believed that in the future, hybrid models coupling continuous and discrete approaches may well come to blur

these frontiers (e.g. Alarcòn, Byrne & Maini, 2004; Cickovski *et al.,* 2005).

VII. Conclusions

1. In the first section, I categorized and emphasized (coarsely I admit) the differences in the questions asked in quite separated fields dealing with morphogenesis *sl*. This section highlighted three main views recognized by the researchers' semantic preference for the terms patterning, pattern formation and morphogenesis, respectively: (**a)**-the first view concentrates on how cells spatiotemporally differentiate and proposes that cells can interpret thresholds along monotonic morphogens gradients in order to specify a unique positional value with respect to some boundaries (positional information). The interpretation of thresholds is assumed to depend on the cells' developmental history and genetic background; (**b)**- the second view focuses on the emergence of spatiotemporal heterogeneities (mainly chemical gradients) and stresses the role of self-organizing dynamics in the establishment of chemical morphogenetic fields; **(c)**- the third view rather places emphasis on how three-dimensional shape comes about with embryonic movements and explores the role of physical forces (mainly continuum mechanics but also chemistry and cell-cell interactions) in the origination of biological form. I emphasized some differences in the concepts of morphogens and morphogenetic fields. These differences are related to the static or dynamic view of morphogenesis that researchers favour.

2. This led me to question what self-organization is, and why it is so uncomfortable to deal with. I tried to point out the different points of view (external *versus* internal observer) and 'causal ideals' underlying how morphogenesis *sl* is understood. As stated by Lewontin (2000a, p. 75), *"[o]ur ignorance of the generation of organic shape also remains profound, despite the progress made by molecular studies of development. What developmental genetics has done is to substitute a question that it can answer for one that it cannot, but without an explicit knowledge of the switch…The question answered by developmental genetics is which genes are being read by the cells at the front end of an embryo and which at the back end. But which genes are read is not an answer to the problem of shape"*. Here we are faced with two incompatible world views: (**a)**-one implying linear causal chains that are mainly located at the molecular- submolecular level, **(b)**- and one implying circular causality (feedbacks) between all levels of organization. The recognition of these differences could help in drawing a clearer picture of how diverse material factors interact to generate biological form. In particular, it should be questioned whether pattern formation and morphogenesis should be considered as two separate questions. The consensus position emerging from the recent improvements of mathematical models of morphogenesis emphasizes the interplay between hierarchical levels of organization and the connection between chemical and mechanical mechanisms.

3. As noticed by Strohman (2000, p. 576), "*the principle of organization as 'cause in the matter' emerges as a dominant theme*". My goal was to discuss a framework (structuralism-dialectics) that, in my opinion, helps in having theory and experimentation working hand by hand. It also assists in describing and understanding counter-intuitive properties of complex systems, especially in the case of morphogenesis *sl* (e.g. homeostasis and homeosis). It seems that the

structuralist-dialectical framework is implicitly endorsed by many, and I think that it would be beneficial that researchers, philosophers and historians of science more explicitly recognize this common foundation. Yet, it is perhaps no need for the word *structuralism* to come again into the play, especially if it reawakens some misunderstandings, such as the structuralist/functionalist debates that pervaded the history of 'evo-devo'.

4. In 2000, Gilbert & Sarkar (p. 8) were noting that *"in developmental biology—one of the birthplaces of complex systems analysis and a field characterized by interacting and emerging systems—computational modeling and analysis have not moved far at all"*. I hope that the ideas discussed in this paper have highlighted the richness and utility of modelling approaches available to us. If the limits of linear thinking have really been amply recognized as many argued around the 2000's, we have to embrace complexity and its fascinating questions.

VIII. References

Alarcòn, T., Byrne, H. M. & Maini, P. K. (2004). Toward whole organ modelling of tumour growth. *Progress in Biophysics and Molecular Biology* **85,** 451-472.

Alberch, P. (1980). Ontogenesis and morphological diversification. *American Zoologist* **20,** 653-667.

Alberch, P. (1989). The logic of monsters: Evidence for internal constraint in development and evolution. In *Ontogénèse et évolution*, vol. 12. *Geobios, Mémoire spécial* (ed. D. B. Dommergues, J. L. Chaline and B. Laurin), pp. 21-57, Lyon.

Alberch, P. (1993). Book reviews: Wolpert, L. The Triumph of the Embryo. Oxford University Press (1991). *Journal of Evolutionary Biology* **6,** 457-459.

Alberch, P. & Gale, E. A. (1985). A developmental analysis of an evolutionary trend: digital reduction in Amphibians. *Evolution* **39,** 8-23.

Aldana, M., Balleza, E., Kauffman, S. & Resendiz, O. (2007). Robustness and evolvability in genetic regulatory networks. *Journal of Theoretical Biology* **245,** 433-448.

Alvarez de Lorenzana, J. M. (1998). Self-organization and self-construction of order. In *Evolutionary Systems: Biological and Epistemological Perspectives on Selection and Self-Organization* (ed. G. Van de Vijver, S. N. Salthe and M. Delpos), pp. 67-78. Kluwer Academic Publishers, Dordrecht.

Amundson, R. (1994). Two concepts of constraint: Adaptationism and the challenge from developmental biology. *Philosophy of Science* **61,** 556-578.

Amundson, R. (1998). Typology reconsidered: Two doctrines on the history of evolutionary biology. *Biology & Philosophy* **13,** 153-177.

Amundson, R. (2005). *The Changing Role of the Embryo in Evolutionary Thought: Roots of Evo-Devo*. Cambridge University Press, Cambridge.

Aranda-Anzaldo, A. (2002). Understanding cancer as a formless phenomenon. *Medical Hypotheses* **59,** 68-75.

Arthur, W. (2004). The effect of development on the direction of evolution: toward a twenty-first century consensus. *Evol Dev* **6,** 282-288.

Ashe, H. L. & Briscoe, J. (2006). The interpretation of morphogen gradients. *Development* **133,** 385-394.

Bard, J. & Lauder, I. (1974). How well does Turing's theory of morphogenesis work? *Journal of Theoretical Biology* **45,** 501-531.

Barrio, R. A., Varea, C., Aragon, J. L. & Maini, P. K. (1999). A two-dimensional numerical study of spatial pattern formation in interacting Turing systems. *Bulletin of Mathematical Biology* **61,** 483-505.

Beloussov, L. V. & Gordon, R. (2006). Developmental morphodynamics - bridging the gap between the genome and embryo physics - Preface. *International Journal of Developmental Biology* **50,** 79-80.

Beloussov, L. V. & Grabovsky, V. I. (2006). Morphomechanics: goals, basic experiments and models. *International Journal of Developmental Biology* **50,** 81-92.

Beloussov, L. V., Louchinskaia, N. N. & Stein, A. A. (2000). Tension-dependent collective cell movements in the early gastrula ectoderm of *Xenopus laevis* embryos. *Development Genes and Evolution* **210,** 92-104.

Ben Amar, M. & Goriely, A. (2005). Growth and instability in elastic tissues. *Journal of the Mechanics and Physics of Solids* **53,** 2284-2319.

Benson, D. L., Maini, P. K. & Sherratt, J. A. (1993). Analysis of pattern formation in reaction-diffusion models with spatially inhomogenous

diffusioncoefficients.*MathematicalandComputer Modelling* **17,** 29-34.

Beysens, D. A., Forgacs, G. & Glazier, J. A. (2000a). Cell sorting is analogous to phase ordering in fluids. *Proceedings of the National Academy of Sciences of the United States of America* **97,** 9467-9471.

Beysens, D. A., Forgacs, G. & Glazier, J. A. (2000b). Embryonic tissues are viscoelastic materials. *Canadian Journal of Physics* **78,** 243-251.

Brouzes, E. & Farge, E. (2004). Interplay of mechanical deformation and patterned gene expression in developing embryos. *Current Opinion in Genetics & Development* **14,** 367-374.

Bunow, B., Kernevez, J.-P., Joly, G. & Thomas, D. (1980). Pattern formation by reaction-diffusion instabilities: Application to morphogenesis in Drosophila. *Journal of Theoretical Biology* **84,** 629-649.

Cai, A. Q., Landman, K. A. & Hughes, B. D. (2007). Multi-scale modeling of a wound-healing cell migration assay. *Journal of Theoretical Biology* **245,** 576-594.

Chaturvedi, R., Izaguirre, J. A., Huang, C., Cickovski, T., Virtue, P., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A. & Glazier, J. A. (2003). Multi-model simulations of chicken limb morphogenesis. In *Computational Science - ICCS 2003, Part III, Proceedings*, vol. 2659. *Lecture Notes in Computer Science*, pp. 39-49. Springer-Verlag Berlin, Berlin.

Cherdantseva, E. M. & Cherdantsev, V. G. (2006). Geometry and mechanics of teleost gastrulation and the formation of primary embryonic axes. *International Journal of Developmental Biology* **50,** 157-168.

Cickovski, T. M., Huang, C. B., Chaturvedi, R., Glimm, T., Hentschel, H. G. E., Alber, M. S., Glazier, J. A., Newman, S. A. & Izaguirre, J. A. (2005). A framework for three-dimensional simulation of morphogenesis. *IEEE-ACM Transactions on Computational Biology and Bioinformatics* **2,** 273-288.

Cocho, G., Perezpascual, R. & Rius, J. L. (1987). Discrete systems, cell-cell interactions and color pattern of animals .1. Conflicting dynamics and pattern formation. *Journal of Theoretical Biology* **125,** 419-435.

Collier, J. R., Monk, N. A. M., Maini, P. K. & Lewis, J. H. (1996). Pattern formation by lateral inhibition with feedback: A mathematical model of Delta-Notch intercellular signalling. *Journal of Theoretical Biology* **183,** 429-446.

Coudreuse, D. & Korswagen, H. C. (2006). Wnt gradient formation: from source to sink. *Animal Biology* **56,** 425-446.

Crampin, E. J., Gaffney, E. A. & Maini, P. K. (1999). Reaction and diffusion on growing domains: Scenarios for robust pattern formation. *Bulletin of Mathematical Biology* **61,** 1093-1120.

Davidson, L. A., Koehl, M. A. R., Keller, R. & Oster, G. F. (1995). How do sea urchins invaginate: Using biomechanics to distinguish between mechanisms of primary invagination. *Development* **121,** 2005-2018.

Davidson, L. A., Oster, G. F., Keller, R. E. & Koehl, M. A. R. (1999). Measurements of mechanical properties of the blastula wall reveal which hypothesized mechanisms of primary invagination are physically plausible in the sea urchin *Strongylocentrotus purpuratus*. *Developmental Biology* **209,** 221-238.

De Jong, H. (2002). Modeling and simulation of genetic regulatory systems: A literature review. *Journal of Computational Biology* **9,** 67-103.

Deutsch, A. & Dormann, S. (2005). *Cellular automaton modeling of biological pattern formation: Characterization, Applications, and Analysis*. Birkhäuser, Boston, Basel, Berlin.

Dillon, R., Maini, P. K. & Othmer, H. G. (1994). Pattern formation in generalized Turing systems .1. Steady state patterns in systems with mixed boundary conditions. *Journal of Mathematical Biology* **32,** 345-393.

Dillon, R. & Othmer, H. G. (1999). A mathematical model for outgrowth and spatial patterning of the vertebrate limb bud. *Journal of Theoretical Biology* **197,** 295-330.

Douady, S. & Couder, Y. (1992). Phyllotaxis as a physical self-organized growth process. *Physical Review Letters* **68,** 2098-2101.

Drasdo, D. & Forgacs, G. (2000). Modeling the interplay of generic and genetic mechanisms in cleavage, blastulation, and gastrulation. *Developmental Dynamics* **219,** 182-191.

Duboule, D. & Wilkins, A. S. (1998). The evolution of 'bricolage'. *Trends in Genetics* **14,** 54-59.

Dudley, A. T., Ros, M. A. & Tabin, C. J. (2002). A re-examination of the proximodistal patterning during vertebrate limb development. *Nature* **418,** 539-544.

Eble, G. J. (1999). On the dual nature of chance in evolutionary biology and paleobiology. *Paleobiology* **25,** 75-87.

Eldar, A., Shilo, B.-Z. & Barkai, N. (2004). Elucidating mechanisms underlying robustness of morphogen gradients. *Current Opinion in Genetics & Development* **14,** 435-439.

Emlen, J. M., Freeman, D. C., Mills, A. & Graham, J. H. (1998). How organisms do the right thing: The attractor hypothesis. *Chaos* **8,** 717-726.

Eungdamrong, N. J. & Iyengar, R. (2004). Computational approaches for modeling regulatory cellular networks. *Trends in Cell Biology* **14,** 661-669.

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Claredon Press, Oxford.

Foty, R., Pfleger, C., Forgacs, G. & Steinberg, M. (1996). Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* **122,** 1611-1620.

Gerhart, J. & Kirschner, M. (1997). *Cells, Embryos and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability*. Blackwell Science, Malden, MA.

Gierer, A. & Meinhardt, H. (1972). Theory of biological pattern formation. *Kybernetik* **12,** 30-39.

Gilbert, S. F. (2000). Diachronic biology meets evo-devo: C. H. Waddington's approach to evolutionary developmental biology. *American Zoologist* **40,** 729-737.

Gilbert, S. F. (2003). The morphogenesis of evolutionary developmental biology. *International Journal of Developmental Biology* **47,** 467-477.

Gilbert, S. F. & Bolker, J. A. (2001). Homologies of process and modular elements of embryonic construction. *Journal of Experimental Zoology* **291,** 1-12.

Gilbert, S. F., Opitz, J. M. & Raff, R. A. (1996). Resynthesizing Evolutionary and Developmental Biology. *Developmental Biology* **173,** 357-372.

Gilbert, S. F. & Sarkar, S. (2000). Embracing complexity: organicism for the 21st century. *Developmental Dynamics* **219,** 1-9.

Glazier, J. A. & Graner, F. (1993). Simulation of the differential adhesion driven rearrangement of biological cells. *Physical Review* **47,** 2128-2154.

Goodwin, B. C. (1988). Problems and prospects in morphogenesis. *Experientia* **44,** 633-637.

Goodwin, B. C. (1994). *How the leopard changed its spots: The evolution of complexity*. Weidenfeld & Nicolson, London.

Goodwin, B. C. (2000). The life of form. Emergent patterns of morphological transformation. *Comptes Rendus de l'Académie des Sciences Série III-Sciences de la Vie-Life Sciences* **323,** 15-21.

Goodwin, B. C. & Trainor, L. E. H. (1985). Tip and whorl morphogenesis in *Acetabularia* by calcium regulated strain fields. *Journal of Theoretical Biology* **117,** 79-106.

Gould, S. J. (1977). *Ontogeny and Phylogeny*. The Belknap Press of Harvard University Press, Cambridge, MA & London.

Gould, S. J. & Lewontin, R. C. (1979). Spandrels of San-Marco and the panglossian paradigm: a critique of the adaptationist program. *Proceedings of the Royal Society of London Series B-Biological Sciences* **205,** 581-598.

Green, J. (2002). Morphogen gradients, positional information, and Xenopus: interplay of theory and experiment. *Developmental Dynamics* **225,** 392-408.

Green, P. B. (1992). Pattern formation in shoots: A likely role for minimal energy configurations of the tunica. *International Journal of Plant Sciences* **153,** S59-S75.

Green, P. B. (1999). Expression of pattern in plants: Combining molecular and calculus-based biophysical paradigms. *American Journal of Botany* **86,** 1059-1076.

Greene, J. C. (1992). From Aristotle to Darwin: Reflections on Ernst Mayr's interpretation in *The Growth of Biological Thought*. *Journal of the History of Biology* **25,** 257-284.

Gursky, V. V., Jaeger, J., Kozlov, K. N., Reinitz, J. & Samsonov, A. M. (2004). Pattern formation and nuclear divisions are uncoupled in Drosophila segmentation: comparison of spatially discrete and continuous models. *Physica D-Nonlinear Phenomena* **197,** 286-302.

Hall, B. K. (1996). *Baupläne*, phylotypic stages, and constraint: Why there are so few types of animals. *Evolutionary Biology* **29,** 215-261.

Hall, B. K. (2000). *Homology: The hierarchical basis of comparative biology*. Academic Press, San Diego, CA.

Hall, B. K. (2003). Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. *Biological Reviews* **78,** 409-433.

Hamburger, V. & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of Morphology* **88,** 49-92.

Hammer, O. & Bucher, H. (1999). Reaction-diffusion processes: Application to the morphogenesis of ammonoid ornamentation. *Geobios* **32,** 841-852.

Harris, A. K., Stopak, D. & Warner, P. (1984). Generation of spatially periodic patterns by a mechanical instability: A mechanical alternative to the Turing Model. *Journal of Embryology and Experimental Morphology* **80,** 1-20.

Harrison, L. G., Wehner, S. & Holloway, D. M. (2001). Complex morphogenesis of surfaces: theory and experiment on coupling of reaction-diffusion patterning to growth. *Faraday Discussions* **120,** 277-294.

Henderson, J. H. & Carter, D. R. (2002). Mechanical induction in limb morphogenesis: the role of growth-generated strains and pressures. *Bone* **31,**

645-653.

Hentschel, H. G. E., Glimm, T., Glazier, J. A. & A., N. S. (2004). Dynamical mechanisms for skeletal pattern formation in the vertebrate limb. *Proceedings of the Royal Society of London B* **271,** 1713-1722.

His, W. (1888). On the principles of animal morphology. *Royal Society of Edinburgh Proceedings* **15,** 287-298.

Hogeweg, P. (2000). Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation. *Journal of Theoretical Biology* **203,** 317-333.

Holtfreter, J. (1939). Gewebeaffinität, ein Mittel der embryonalen Formbildung. *Archiv für experimentelle Zellforschung, besonders Gewebezüchtung* **23,** 169-209.

Horder, T. J. (1993). The chicken and the egg. In *Experimental and Theoretical Advances in Biological Pattern Formation*, vol. 259 (ed. H. G. Othmer, P. K. Maini and J. D. Murray), pp. 121-148. Plenum Press. NATO ASI Series. Series A: Life Sciences, New York, London.

Horder, T. J. (2001). The organizer concept and modern embryology: Anglo-American perspectives. *International Journal of Developmental Biology* **45,** 97-132.

Horder, T. J. (2006). Gavin Rylands de Beer: how embryology foreshadowed the dilemmas of the genome. *Nature Reviews Genetics* **7,** 892-898.

Huang, S. & Ingber, D. E. (2000). Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Experimental Cell Research* **261,** 91-103.

Ingber, D. E. (2002). Cancer as a disease of epithelial-mesenchymal interactions and extracellular matrix regulation. *Differentiation* **70,** 547-560.

Ingber, D. E. (2006a). Cellular mechanotransduction: putting all the pieces together again. *FASEB journal* **20,** 811-827.

Ingber, D. E. (2006b). Mechanical control of tissue morphogenesis during embryological development. *International Journal of Developmental Biology* **50,** 255-266.

Izaguirre, J. A., Chaturvedi, R., Huang, C., Cickovski, T., Coffland, J., Thomas, G., Forgacs, G., Alber, M., Hentschel, G., Newman, S. A. & Glazier, J. A. (2004). CompuCell, a multi-model framework for simulation of morphogenesis. *Bioinformatics* **20,** 1129-1137.

Jaeger, J., Blagov, M., Kosman, D., Kozlov, K. N., Manu, Myasnikova, E., Surkova, S., Vanario-Alonso, C. E., Samsonova, M., Sharp, D. H. & Reinitz, J. (2004). Dynamical analysis of regulatory interactions in the gap gene system of *Drosophila melanogaster*. *Genetics* **167,** 1721-1737.

Jaeger, J. & Goodwin, B. C. (2001). A cellular oscillator model for periodic pattern formation. *Journal of Theoretical Biology* **213,** 171-181.

Jaeger, J. & Reinitz, J. (2006). On the dynamic nature of positional information. *BioEssays* **28,** 1102-1111.

Jenner, R. A. (2006). Unburdening evo-devo: ancestral attractions, model organisms, and basal baloney. *Development Genes and Evolution* **216,** 385-394.

Kauffman, S. A. (1993). *The Origin of Order: Self-organization and Selection in Evolution*. Oxford University Press, New York.

Kauffman, S. A., Shymko, R. M. & Trabert, K. (1978). Control of sequential compartment formation in *Drosophila*. *Science* **199,** 259-270.

Keller, R., Davidson, L. A. & Shook, D. R. (2003). How we are shaped: the biomechanics of gastrulation. *Differentiation* **71,** 171-205.

Kiskowski, M. A., Alber, M. S., Thomas, G. L., Glazier, J. A., Bronstein, N. B., Pu, J. & Newman, S. A. (2004). Interplay between activator-inhibitor coupling and cell-matrix adhesion in a cellular automaton model for chondrogenic patterning. *Developmental Biology* **271,** 372-387.

Kondo, S. & Asai, R. (1995). A reaction-diffusion wave on the skin of the marine angelfish *Pomacanthus*. *Nature* **376,** 765-768.

Lehman, H. E. (1957). The developmental mechanics of pigment pattern formation in the black Axolotl, *Amblystoma Mexicanum*. 1. the formation of yellow and black bars in young larvae. *Journal of Experimental Zoology* **135,** 355-386.

Levins, R. & Lewontin, R. (1980). Dialectics and reductionism in ecology. *Synthese* **43,** 47-78.

Levins, R. & Lewontin, R. C. (1985). *The Dialectical Biologist*. Harvard University Press, Cambridge, MA & London.

Lewontin, R. C. (2000a). *It ain't necessarily so: the dream of the human genome and other illusions*. New York : New York Review of Books.

Lewontin, R. C. (2000b). *The triple helix: gene, organism, and environment*. Harvard University Press, Cambridge, MA.

Love, A. C. (2003). Evolutionary morphology, innovation, and the synthesis of evolutionary and developmental biology. *Biology & Philosophy* **18,** 309-345.

Maini, P. K. (1996). Spatial and spatiotemporal pattern formation in generalised Turing systems. *Computers*

& Mathematics with Applications **32,** 71-77.

Maini, P. K. (2003). How the mouse got its stripes. *Proceedings of the National Academy of Sciences of the United States of America* **100,** 9656-9657.

Malygin, A. G. (2006). Morphodynamics of phyllotaxis. *International Journal of Developmental Biology* **50,** 277-287.

Marée, A. F. M. (2000). From pattern formation to morphogenesis: multicellular coordination in *Dictyostelium discoideum*, Utrecht University.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. & Wolpert, L. (1985). Developmental constraints and evolution: a perspective from the Mountain Lake conference on development and evolution. *Quarterly Review of Biology* **60,** 265-287.

Mayr, E. (1961). Cause and effect in biology. *Science* **134,** 1501-1506.

Mayr, E. (1963). *Animal species and evolution.* Oxford University Press, Cambridge.

Meinhardt, H. (1992). Pattern formation in biology: a comparison of models and experiments. *Reports on Progress in Physics* **55,** 797-849.

Meinhardt, H. (1995). *The algorithmic beauty of sea shells*. Springer-Verlag, Berlin.

Meinhardt, H. (2000). Models for organizer and notochord formation. *Comptes Rendus de l'Académie des Sciences Série III-Sciences de la Vie-Life Sciences* **323,** 23-30.

Meinhardt, H. (2003). Complex pattern formation by a self-destabilization of established patterns: chemotactic orientation and phyllotaxis as examples. *Comptes Rendus Biologies* **326,** 223-237.

Meinhardt, H. & Gierer, A. (2000). Pattern formation by local self-activation and lateral inhibition. *BioEssays* **22,** 753-760.

Miura, T. & Maini, P. K. (2004). Speed of pattern appearance in reaction-diffusion models: implications in the pattern formation of limb bud mesenchyme cells. *Bulletin of Mathematical Biology* **66,** 627-649.

Miura, T. & Shiota, K. (2000). TGF beta 2 acts as an "activator" molecule in reaction-diffusion model and is involved in cell sorting phenomenon in mouse limb micromass culture. *Developmental Dynamics* **217,** 241-249.

Miura, T. & Shiota, K. (2002). Depletion of FGF acts as a lateral inhibitory factor in lung branching morphogenesis *in vitro*. *Mechanisms of Development* **116,** 29-38.

Miura, T., Shiota, K., Morriss-Kay, G. & Maini, P. K. (2006). Mixed-mode pattern in Doublefoot mutant mouse limb — Turing reaction-diffusion model on a growing domain during limb development. *Journal of Theoretical Biology* **240,** 562-573.

Monk, N. A. M. (2000). Elegant hypothesis and inelegant fact in developmental biology. *Endeavour* **24,** 170-173.

Morita, R. (1991). Finite element analysis of a double membrane tube (DMS-tube) and its implication for gastropod shell morphology. *Journal of Morphology* **207,** 81-92.

Müller, G. B. & Newman, S. A. (2003). *Origination of organismal form: beyond the gene in developmental and evolutionary biology*. MIT Press, Cambridge, Mass.

Murray, J. D. (1989). *Mathematical Biology*. Springer-Verlag, Berlin, Heidelberg.

Murray, J. D. (2000). Pattern formation in integrative biology — a marriage of theory and experiment. *Comptes Rendus de l'Académie des Sciences Série III-Sciences de la Vie-Life Sciences* **323,** 5-14.

Nagorcka, B. N. (1988). A pattern formation mechanism to control spatial organization in the embryo of *Drosophila melanogaster*. *Journal of Theoretical Biology* **132,** 277-306.

Neagu, A., Jakab, K., Jamison, R. & Forgacs, G. (2005). Role of physical mechanisms in biological self-organization. *Physical Review Letters* **95**, 178104.

Nelson, C. M., Jean, R. P., Tan, J. L., Liu, W. F., Sniadecki, N. J., Spector, A. A. & Chen, C. S. (2005). Emergent patterns of growth controlled by multicellular form and mechanics. *Proceedings of the National Academy of Sciences of the United States of America* **102,** 11594-11599.

Newman, S. A. & Frisch, H. L. (1979). Dynamics of skeletal pattern formation in developing chick limb. *Science* **205,** 662-668.

Newman, S. A. & Muller, G. B. (2005). Origination and innovation in the vertebrate limb skeleton: an epigenetic perspective. *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution* **304B,** 593-609.

Nijhout, H. F. (1990). Metaphors and the role of genes in development. *BioEssays* **12,** 441-446.

Nijhout, H. F., Maini, P. K., Madzvamuse, A., Wathen, A. J. & Sekimura, T. (2003). Pigmentation pattern formation in butterflies: experiments and models. *Comptes Rendus Biologies* **326,** 717-727.

Odell, G., Oster, G., Burnside, B. & Alberch, P. (1980). A mechanical model for epithelial morphogenesis. *Journal of Mathematical Biology* **9,** 291-295.

Olsson, L. & Löfberg, J. (1993). Pigment cell migration and pattern formation in salamander larvae. In *Oscillations and morphogenesis* (ed. L. Rensing),

pp. 453-462. Marcel Dekker, New York.

Osborn, J. W. (1993). A model simulating tooth morphogenesis without morphogens. *Journal of Theoretical Biology* **165,** 429-445.

Oster, G. F., Odell, G., Burnside, B. & Alberch, P. (1979). Mechanical Model for Epithelial Morphogenesis and Certain Gastrulation Patterns. *American Zoologist* **19,** 1014-1014.

Oster, G. F. & Odell, G. M. (1984). The mechanochemistry of cytogels. *Physica D* **12,** 333-350.

Oster, G. F., Shubin, N., Murray, J. D. & Alberch, P. (1988). Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. *Evolution* **42,** 862-884.

Palsson, E. (2001). A three-dimensional model of cell movement in multicellular systems. *Future Generation Computer Systems* **17,** 835-852.

Piaget, J. (1972). *Le structuralisme*, Fifth edition. Presses universitaires de France, Paris.

Plahte, E. (2001). Pattern formation in discrete cell lattices. *Journal of Mathematical Biology* **43,** 411-445.

Podos, S. D. & Ferguson, E. L. (1999). Morphogen gradients: New insights from DPP. *Trends in Genetics* **15,** 396-402.

Poplawski, N. J., Swat, M., Gens, J. C. & Glazier, J. A. (2007). Adhesion between cells, diffusion of growth factors, and elasticity of the AER produce the paddle shape of the chick limb. *Physica A* **373,** 521-532.

Raff, R. A. & Kaufman, T. C. (1983). *Embryos, genes, and evolution : the developmental-genetic basis of evolutionary change*. Macmillan, Collier Macmillan, New York, London.

Reeves, G. T., Muratov, C. B., Schupbach, T. & Shvartsman, S. Y. (2006). Quantitative models of developmental pattern formation. *Developmental Cell* **11,** 289-300.

Rieppel, O. (1990). Structuralism, functionalism, and the four Aristotelian causes. *Journal of the History of Biology* **23,** 291-320.

Rubin, H. (2006). What keeps cells in tissues behaving normally in the face of myriad mutations? *BioEssays* **28,** 515-524.

Salazar-Ciudad, I. (2006a). Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development? *Journal of Experimental Zoology Part B - Molecular and Developmental Evolution* **306B,** 107-125.

Salazar-Ciudad, I. (2006b). On the origins of morphological disparity and its diverse developmental bases. *BioEssays* **28,** 1112-1122.

Salazar-Ciudad, I., Garcia-Fernandez, J. & Sole, R. V. (2000). Gene networks capable of pattern formation: From induction to reaction-diffusion. *Journal of Theoretical Biology* **205,** 587-603.

Salazar-Ciudad, I. & Jernvall, J. (2002). A gene network model accounting for development and evolution of mammalian teeth. *Proceedings of the National Academy of Sciences of the United States of America* **99,** 8116-8120.

Salazar-Ciudad, I. & Jernvall, J. (2004). How different types of pattern formation mechanisms affect the evolution of form and development. *Evolution & Development* **6,** 6-16.

Salazar-Ciudad, I., Jernvall, J. & Newman, S. A. (2003). Mechanisms of pattern formation in development and evolution. *Development* **130,** 2027-2037.

Salazar-Ciudad, I., Newman, S. A. & Sole, R. V. (2001). Phenotypic and dynamical transitions in model genetic networks I. Emergence of patterns and genotype-phenotype relationships. *Evolution & Development* **3,** 84-94.

Salazar-Ciudad, I., Sole, R. V. & Newman, S. A. (2001). Phenotypic and dynamical transitions in model genetic networks II. Application to the evolution of segmentation mechanisms. *Evolution & Development* **3,** 95-103.

Sato, K., Koizumi, Y., Takahashi, M., Kuroiwa, A. & Tamura, K. (2007). Specification of cell fate along the proximal-distal axis in the developing chick limb bud. *Development* **134,** 1397-1406.

Schiffmann, Y. (1991). An hypothesis: Phosphorylation fields as the source of positional information and cell differentiation: (cAMP, ATP) as the universal morphogenetic Turing couple. *Progress in Biophysics & Molecular Biology* **56,** 79-105.

Smith, J. C. & Wolpert, L. (2000). From engineering to positional information to public understanding - An interview with Lewis Wolpert. *International Journal of Developmental Biology* **44,** 85-91.

Soto, A. M. & Sonnenschein, C. (2005). Emergentism as a default: Cancer as a problem of tissue organization. *Journal of Biosciences* **30,** 103-118.

Steinberg, M. S. (1970). Does differential adhesion govern self-assembly processes in histogenesis: Equilibrium configurations and emergence of a hierarchy among populations of embryonic cells. *Journal of Experimental Zoology* **173,** 395-433.

Stemple, D. L. & Vincent, J. P. (2004). Pattern formation and developmental mechanisms: Super-models of development. *Current Opinion in Genetics and Development* **14,** 325-327.

Strohman, R. C. (1997). The coming Kuhnian revolution in biology. *Nature Biotechnology* **15,** 194-200.

Strohman, R. C. (2000). Organization becomes cause in the matter. *Nature Biotechnology* **18,** 575-576.

Tabata, T. & Takei, Y. (2004). Morphogens, their identification and regulation. *Development* **131,** 703-712.

Theise, N. D. (2006). Implications of 'postmodern biology' for pathology: the cell doctrine. *Laboratory Investigation* **86,** 335-344.

Thom, R. (1972). *Stabilité structurelle et morphogenèse: essai d'une théorie générale des modèles*. Reading MA, Benjamin.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Tickle, C. (2003). Patterning systems: from one end of the limb to the other. *Developmental Cell* **4,** 449-458.

Tracqui, P. (2006). Mechanical instabilities as a central issue for *in silico* analysis of cell dynamics. *Proceedings of the IEEE* **94,** 710-724.

Tracqui, P. & Ohayon, J. (2004). Transmission of mechanical stresses within the cytoskeleton of adherent cells: a theoretical analysis based on a multicomponent cell model. *Acta Biotheoretica* **52,** 323-341.

Trainor, P. (2003). Vertebrate patterning: how to cross-stitch an embryo. *Development* **130,** 5561-5562.

Tranqui, L. & Tracqui, P. (2000). Mechanical signalling and angiogenesis. The integration of cell-extracellular matrix couplings. *Comptes Rendus de l'Académie des Sciences Série III-Sciences de la Vie-Life Sciences* **323,** 31-47.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **237,** 37-72.

Urdy, S. & Chirat, R. (2006). Snail shell coiling (re-) evolution and the evo-devo revolution. *Journal of Zoological Systematics and Evolutionary Reasearch* **44,** 1-7.

Van de Vijver, G., Van Speybroeck, L. & Vandevyvere, W. (2003). Reflecting on complexity of biological systems: Kant and beyond? *Acta Biotheoretica* **51,** 101-140.

Van der Weele, C. (1993). Explaining embryological development: should integration be the goal? *Biology & Philosophy* **8,** 385-397.

Varea, C., Aragon, J. L. & Barrio, R. A. (1997). Confined Turing patterns in growing systems. *Physical Review E* **56,** 1250-1253.

Vincent, J. P. & Briscoe, J. (2001). Morphogens. *Current Biology* **11,** R851-R854.

Waddington, C. H. (1947). *Organisers and genes*. Cambridge University Press, Cambridge.

Waddington, C. H. (1956). *Principles of Embryology*. Allen and Unwin, London.

Waddington, C. H. (1977). Stabilisation in systems: chreods and epigenetic landscapes. *Futures* **9,** 139.

Wake, D. B. (1991). Homoplasy: the result of natural selection, or evidence of design limitations? *American Naturalist* **138,** 543-567.

Wang, J. & Thampatty, B. (2006). An introductory review of cell mechanobiology. *Biomechanics and Modeling in Mechanobiology* **5,** 1-16.

Webster, G. & Goodwin, B. C. (1982). The origin of species: a structuralist approach. *Journal of Social and Biological Structures* **5,** 15-47.

Weliky, M., Minsuk, S., Keller, R. & Oster, G. (1991). Notochord morphogenesis in *Xenopus laevis*: simulation of cell behavior underlying tissue convergence and extension. *Development* **113,** 1231-1244.

Weliky, M. & Oster, G. (1990). The mechanical basis of cell rearrangement .1. Epithelial morphogenesis during *Fundulus* epiboly. *Development* **109,** 373-386.

Whitehead, A. N. (1919). *The concept of nature*. Tarner Lectures. Cambridge University Press, Cambridge.

Wilby, O. K. & Ede, D. A. (1975). Model generating pattern of cartilage skeletal elements in embryonic chick limb. *Journal of Theoretical Biology* **52,** 199-217.

Wimsatt, W. C. (2006). Aggregate, composed, and evolved systems: reductionistic heuristics as means to more holistic theories. *Biology & Philosophy* **21,** 667-702.

Wolpert, L. (1969). Positional information and spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25,** 1-47.

Wolpert, L. (1996). One hundred years of positional information. *Trends in Genetics* **12,** 359-364.

Wolpert, L. & Hornbruch, A. (1990). Double anterior chick limb buds and models for cartilage rudiment specification. *Development* **109,** 961-966.

Wolpert, L., Smith, J., Jessell, T. M., Lawrence, P., Robertson, E. & Meyerowitz, E. (2006). *Principles of development*. Oxford University Press, Oxford.

Yamaguchi, M., Yoshimoto, E. & Kondo, S. (2007). Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 4790-4793.

Young, B. A. (1993). On the necessity of an archetypal concept in morphology: with special reference to the concepts of "structure" and "homology". *Biology & Philosophy* **8,** 225-248.

Book review - Principles of morphogenesis: the contribution of cellular automata models

Review of:
Deutsch, A. and Dormann, S. 2005. Cellular automaton modelling of biological pattern formation: Characterization, Applications, and Analysis. Modeling and Simulation in Science, Engineering and Technology. N. Bellomo (Eds). Birkhäuser, Boston, Basel, Berlin. 331 p. Price 89.95 USD

Reference: Acta Zoologica (Stockholm) **90**: 205–208 (April 2009)

Contents

I. Introduction

Cellular automata (CA) models have been used in a variety of contexts in physics, chemistry and biology. Such models are particularly well suited to the modelling of local interactions between particles, molecules, cells or individuals. CA represent discrete agents, which occupy some sites of a regularly defined lattice (of a given geometry with boundary condition settings). These agents have one or more internal discrete state variables and the evolution of their state and/or their position on the lattice is described by local rules of interaction between each CA and its neighbourhood (which defines the range of interaction). Compared to continuous approaches, CA models look quite instinctive and easy to implement (at least at first glance). CA can be used when macroscopic 'master equations' are unknown or when the number of interacting agents is simply too low for an accurate continuous approximation of the phenomenon under study.

These abstract models focus exclusively on interaction, regardless of the particular material nature of the agents. This provides such models with a kind of universality that allows

the discovery of generic rules that one can apply in unrelated contexts. For example, the predator-prey models in population studies share strong similarities with activator-inhibitor models used in chemistry and biological pattern formation. Nevertheless, CA approaches also have limitations, which include the difficulty of going from qualitative to quantitative descriptions (measures of dynamics such as entropy and complexity) and of predicting the possible solutions analytically. Because of their abstraction, their apparent simplicity and their relatively 'weak' predictive power, CA models look fascinating to some of their defenders, especially when the simplicity of generative rules are compared to the apparent 'complexity' of the resulting outcomes (Wolfram, 2002). This author even argued that the algorithm-based models such as CA should replace the traditional continuous equation-based models to create a 'new kind of science'.

But, such a generality can also be viewed as an Achilles heel. CA models are often perceived from the outside as mere descriptive computational tools of low explanatory power in opposition to classical analytical mathematical approaches (such as ordinary/partial differential equations, see Ermentrout & Edelstein Keshet, 1993 for review). In the 1940s, the first CA were motivated by the problem of self-reproduction (Von Neumann, 1966) and the building of universal computing machines (Turing machines). These exciting moments of discovery focused more on the utility of CA models as a paradigm of universal computation rather than on the search for an analytic characterization of the solutions. In 2008, CA approaches are widespread in artificial life applications, a domain where prediction does not really appear possible or even wished for. Continuous and discrete traditions have followed separate roads as their goals have diverged: continuous methods rather focused on prediction ('explanation') of 'simplistic tractable models' while discrete methods mainly concentrated on the exploration ('observation') of a wider range of possible dynamics not necessarily simplified (and hence often intractable from an analytical point of view).

However, the frontier between both traditions does not appear as sharp as one can conceive at first sight. Predictive tools for CA exist. Thus, these models are not necessarily aimed at 'simply' reproducing the patterns we observe in nature and nor are they *per se* unable to provide interesting insights into the phenomenon under study. This is nicely exemplified in the book *Cellular Automaton Modelling of Biological Pattern Formation: Characterization, Applications, and Analysis* by Andreas Deutsch and Sabine Dormann.

Apparently, this book seeks to: (1) underline the recent efforts invested in the theoretical foundation of the CA, especially the exploration of the predictive tools for CA analysis; (2) improve the biological explanations through experimental and theoretical cross fertilization.

The book is organized into three main sections. After describing the principles of self-organization and mathematical approaches to pattern formation and morphogenesis (Part I), Deutsch & Dormann review the CA modelling basics and their recent improvements (Part II), and finally give a quite exhaustive overview of several key biological applications (Part III). They try to bridge the gap between theoretical and experimental works in a comprehensible way. I will successively review these contributions and then return to their historical review and indicate briefly some problematic conceptual issues regarding 'self-organization'.

II. Characterization and analysis of CA models

After introducing the goals of CA models, their biological roots and their formal logic, Deutsch & Dormann (p. 79, Chapter 4) recall that *"two basic questions are underlying the analysis of cellular automata…:*

1. How can global behavior be predicted from the knowledge of local rules? (bottom-up approach)

2. How do specific local rules have to be designed in order to yield a preselected global behavior? (top-down approach, inverse problem)".

Deutsch & Dormann are particularly concerned with the first question so they investigate in depth the tools to characterize the analytic solutions of CA (mean field approximation, linear stability analysis, Boltzmann propagator). They then present and discuss the available methods and appropriate conditions to take "the continuum limit", thus linking CA to macroscopic partial differential equation in several instances (space and time scaling). The authors cover deterministic and probabilistic CA, which are, respectively, rooted in Newtonian mechanics and 'Boltzmannian' statistical mechanics.

Probabilistic CA should receive more and more attention in the future since they could greatly improve our understanding of the relationships between the microscopic, mesoscopic and macroscopic levels of biological organization (molecular/cellular/tissue levels) and assist in tackling the intriguing combination of robustness and variability observed in self-organized biological systems. In such probabilistic dynamical models, the same initial conditions will not yield strictly identical results, except for the averaging of a sufficiently large number of simulations. The authors particularly develop the study of Lattice Gas Cellular Automata (LGCA) for these somewhat 'untraditional' CA models are well adapted to simulate random processes of interaction and movement that appear especially widespread in biological applications. Indeed, Deutsch & Dormann illustrate that the combination of interaction and movement in (probabilistic) LGCA models favours their analytical treatment by means of approximation models (lattice-Boltzmann equation).

They make valuable efforts to bring some particular models to detailed comprehensive analytic study using such methods (especially in Chapters 5 and 11). Throughout the book, Deutsch & Dormann (p. 257) convincingly demonstrate that *"CA are neither a replacement for traditional (continuous) mathematical models nor preliminary mathematical models but constitute a proper class of discrete mathematical models: discrete in space, time and state space, for which analytical tools already exist or can be developed in the future"*. This endeavour is viewed as an inevitable requirement if CA approaches are to provide a more and more powerful analytic tool in the future and are to favour a constructive dialogue between theoreticians and experimentalists.

III. Key biological applications

Deutsch & Dormann (p. 6-7, emphasis in the original) expose their biological motivation in these terms: *"Morphogenesis results from a limited repertoire of cellular activities: in particular, cells can change their shape, grow, divide, differentiate, undergo apoptosis, and migrate. It is the core of biological morphogenesis that cells do not behave independently of each other. To the contrary, cellular activities are intertwined*

and strongly rely on cooperative dynamics of ***cell-cell interaction****, which may induce changes in cellular properties and activities…The question is, what are essential cell interactions and how do corresponding cooperative phenomena influence organismic morphogenesis? Possible answers can be found by means of mathematical modeling, which allows one to abstract from specific component behavior and to analyze generic properties"*.

This is what the main part of the book (indeed, two thirds of it) proposes to highlight, by describing detailed applications of high biological relevance: cell aggregation (Chapter 5), cell proliferation (Chapter 6), cell sorting and engulfment (Chapter 7), cell alignment (Chapter 8), pigment cell pattern formation (Chapter 9), tumour growth (Chapter 10) and chemical concentration fields (Chapter 11). The essential principles of interaction reviewed throughout these chapters include random diffusion and diffusion limited aggregation (Chapter 5), cell multiplication and active cell migration (Chapters 6 and 10), differential adhesion (Chapters 7 and 10), orientation induced interaction (Chapter 8), contact guidance or topographic guidance through the extracellular matrix (Chapters 9 and 10), chemotaxis (Chapter 10) and finally Turing instabilities and excitable media (Chapter 11).

These interactions can be viewed as basic 'sets of rules' which can be further combined (as exemplified in Chapter 10) when experimental data suggest theoretical refinements. It is worth noting that the part of the book devoted to applications does not appear as a mere catalogue of unrelated examples because the authors carefully draw the link between the different cases, especially in the final discussion section (Chapter 12). Although each chapter of the book can be read independently, understanding the similarities and differences between the discussed CA models obviously helps to rapidly broaden the picture. Readers will further benefit from the research projects that Deutsch & Dormann suggest at the end of each chapter. Valuable inspiration to support teaching material or to further apply CA approaches or hybrid models to other specific contexts may be found in these sections.

The book by Deutsch & Dormann covers the cellular level well. Although some of the discrete models presented already have continuous equivalents in the literature (e.g. Chapters 7, 9, 10, 11), the authors go beyond merely reproducing already available results. For example, they show that their CA model of the 'differential adhesion hypothesis' (Steinberg, 1970) makes it possible to take active cell migration into account, or to consider asymmetric adhesion. This book will undoubtedly assist in bridging some gaps between experimentalists and theoreticians thanks to a well-balanced presentation of concepts, theories and applications.

However, Deutsch & Dormann (p. 29) note *"that the cellular automaton models introduced in this book focus on interactions. The precise (material) character of the interacting entities is of far less importance, if any. The systems studied are defined by their interactions"*. The fact that CA models are relational models could rather seem unnatural to experimentalists whose favoured type of explanation usually focus on material entities. In general, the questions addressed by experimentalists only superficially match those of theoreticians, illustrating quite different concerns and ways of thinking in spite of apparently similar language. Overlooking these differences could limit the communication between experimentalists and theoreticians. In this book, the link between theory and experiment is efficiently established. For example, the

chapter on pigment pattern formation in larval salamanders compares theoretical with experimental results (e.g. Olsson & Löfberg, 1992). Also, individual cell data, which can be obtained from *in vitro* studies, provide an interesting avenue of research favouring the intertwining between theory and experiment. As Deutsch & Dormann (p.258) write, *"[i]t is a challenge for the future to systematically link pattern formation models as presented in this book to intracellular genetic and signaling networks. This implies covering a whole range of cellular and molecular scales and will hopefully be possible in the future since the experimental data needed for the mathematical modeling already exists or can now be collected"*.

IV. Principles, theories and models of biological organization: some conceptual problems

To me, the brief historical account opening the book leaves something to be desired. This chapter attempts to explain *"how a particular spatio-temporal conception directs possible principles of pattern formation, particularly preformation, optimization, and self-organization"* (p.43). In particular, it equates the concept of preformation of the 19th century (and earlier) with the pre-pattern concept of some modern theories of development. For example, Deutsch and Dormann (p. 35-36) write that the *"[g]enetic program and the notion of locus-responsive control genes (pattern genes) are modern transcriptions of an old concept, preformation. In contrast, regulative development implies that structure is not fully specified in the DNA code: it arises later and more indirectly from changes in the properties of cells and tissues; in other words embryogenesis per se is assumed to play a crucial role in pattern formation"*.

For these reasons, the authors claim that Wolpert's scheme (1969) and reaction-diffusion models (Turing, 1952) are preformationist, insofar as they suppose a 'pre-pattern' from which a specific morphological structure is assumed to be derived. It is true that Wolpert's positional information concept is clearly Weismannist in that it relies on a "central directing agency" (Webster & Goodwin, 1982). As such, it can be recognized as an antonym of 'self-organization'. As noted by Deutsch & Dormann (p. 30), *"[i]n self-organized systems there is no dichotomy between the organizer and the organized"*. However, Wolpert's scheme cannot simply be labelled preformationist, a notion historically restricted to the *homunculus* and which would render any study of embryology unnecessary. Wolpert's scheme and reaction-diffusion models are better characterized as 'morphostatic' models, because they assume that cell-cell signalling proceeds before (and independently of) other cell behaviours (Salazar-Ciudad, Jernvall & Newman, 2003).

More surprisingly, Deutsch & Dormann claim that D'Arcy Thompson's theory of form transformation (1952) is also preformationist. Relying extensively on Horder (1993), they write (p. 39-40): *"Thompson tried to explain form and evolutionary change of form as a result of the immediate, primarily mechanical forces operating on the developing embryo and developed a theory of allometric transformations. Changing morphologies are explained solely as the result of coordinated differential growth during development (preformation concept)"*. However, quantitative descriptions of macroscopic shape changes (like Thompson's transformation grids) can hardly be viewed as a negation of self-organization principles, simply because they

emphasize smooth changes rather than 'bifurcations'. This point may illustrate the authors' bias towards discreteness, their favoured level of explanation (groups of cells) and their restrictive definition of self-organization. The dichotomy between discreteness and continuousness is to a large extent relative to the level of investigation. Are the laws of macroscopic mechanics not the most trivial manifestation of self-organization?

The first part of the book would have deserved more clarity and caution. However, the few points stressed above do not have much consequence for the remaining parts of the book. Inconsistencies in the definitions of preformation and self-organization mainly reflect our difficulties in dealing with the 'morphogenesis issue' in pure literal form, because most of these historically charged words are no longer suited to the explanation of the basic principles of morphogenesis. Actually, the weaknesses mentioned are not at all particular to Deutsch & Dormann's contribution, but can be seen as typical of the whole field. This is not only a 'semantic' issue, as one may think at first glance, but also a conceptual and practical problem. Developmental systems span a hierarchy of scales of interaction and we do not yet have a clear framework for the representation of causality within such systems.

V. Conclusion

Deutsch & Dormann's book begins with an epitaph quoting Albert Einstein, according to which *"things should be made as simple as possible, but not any simpler"*. Undoubtedly, the authors' explicit aim is reached successfully: the content is dense but accessible to a broad audience (including students); the presentation is self-contained despite the small format (handbook). The book provides sufficient guidelines to allow the suitable expansion of CA approaches (3D CA, Cellular Pott models) and their application to other exciting problems in biology. Mathematical models and CA in particular are sometimes blamed for being too general, so that they do not prove useful for the understanding of any specific application. This is not the case here, as the book highlights the most fundamental principles of cell-cell interactions (such as differential adhesion), while providing the constraints (initial and boundary conditions) thanks to which, one can get in touch with what is really going on in particular cases.

Cellular automaton models, among other theoretical approaches, definitively appear as a valuable tool to improve the power of biological explanations. Deutsch & Dorman's book is *"aimed at researchers, practitioners, and students in applied mathematics, mathematical biology, computational biology, computational physics, bioengineering, and computer science interested in a cellular approach to biological modeling"*. But as pointed out by Maini (p. viii) in the Preface, this book is also of interest to the experimentalist as an introduction to mathematical modelling of pattern formation and morphogenesis. Indeed, Deutsch & Dormann's book will receive attention from those recognizing that complexity sciences offer wonderful platforms of discussion between theoreticians and empiricists.

Readers interested in cell/tissue level investigation of morphogenesis will still prefer the now classical *Mathematical biology* by Murray (1989) although the latter book does not cover morphogenesis exclusively. Readers interested in relating 'individual cell data' to theoretical cell models and/or explicit modelling of cell division, migration or deformation will preferably benefit from the discrete approach developed in Deutsch & Dormann's book.

VI. References

Ermentrout, G. B. & Edelstein Keshet, L. (1993). Cellular automata approaches to biological modeling. *Journal of Theoretical Biology* **160,** 97-133.

Horder, T. J. (1993). The chicken and the egg. In *Experimental and Theoretical Advances in Biological Pattern Formation*, vol. 259. *Series A: Life Science* (ed. H. G. Othmer, P. K. Maini and J. D. Murray), pp. 121-148. Plenum Press, New York, London.

Murray, J. D. (1989). *Mathematical Biology*. Springer-Verlag, Berlin, Heidelberg.

Olsson, L. & Lofberg, J. (1992). Pigment pattern formation in larval Ambystomatid salamanders *Ambystoma tigrinum tigrinum*. *Journal of Morphology* **211,** 73-85.

Salazar-Ciudad, I., Jernvall, J. & Newman, S. A. (2003). Mechanisms of pattern formation in development and evolution. *Development* **130,** 2027-2037.

Steinberg, M. S. (1970). Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and emergence of a hierarchy among populations of embryonic cells. *Journal of Experimental Zoology* **173,** 395-433.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **237,** 37-72.

Von Neumann, J. (1966). The Theory of Self-reproducing Automata (ed. A. W. Burks). University of Illinois Press, Urbana.

Webster, G. & Goodwin, B. C. (1982). The origin of species: a structuralist approach. *Journal of Social and Biological Structures* **5,** 15-47.

Wolfram, S. (2002). *A New Kind of Science*. Wolfram Media, Inc., Champaign.

Wolpert, L. (1969). Positional information and spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25,** 1-47.

Chapter 2 - Snail shell coiling (re-)evolution and the evo-devo revolution

Reference: Urdy, S. and Chirat, R. 2006. Snail shell coiling (re-)evolution and the evo-devo revolution. *Journal of Zoological Systematics and Evolutionary Research*. 44(1): 1-7.

Abstract

During the last two decades evolutionary developmental biology has become a major research program whose findings put into question some concepts lying at the core of the 'Synthetic Theory'. However, some authors are waiting for a 'revolution' in biology, one in which the existing genetic determinism will give way to a new conceptual understanding of the complexity of living organisms. This 'revolution' should necessarily pass through the elaboration of an appropriate theoretical framework integrating the non-linear dynamics of development as its fundamental basis. This objective implies a drastic shift in the way causality is generally understood as well as a purge of numerous convenient but misleading metaphors such as genetic or developmental programs. Although most authors do not take these metaphors too literally, some persist to employ such 'instructionist' notions in a more literal perspective, and, in doing so, deny some concepts at the core of evolutionary developmental biology. We critically review two recent studies suggesting that shell coiling has re-evolved in a family of limpets (Calyptraeidae, Gastropoda). We stress that this putative re-evolution of snail shell coiling results only from an arbitrary scoring procedure leading to consider shell coiling as a binary discrete character. We show that the way in which these authors connect this case study to evolutionary theories stems from the unwarranted premise of a linear mapping of genes onto phenotypes where particulate inheritance of morphological characters seems implicitly assumed. We illustrate how the persisting unclear role of genes in morphogenesis allows the maintenance of the adaptationist program.

Key words: evo-devo – complexity – generic physical properties – morphology – genetic determinism – gastropoda – coiling.

Contents

I. Introduction

Over the last twenty years, evolutionary developmental biology has become a major research program. This interdisciplinary approach is focused on how changes in development bring about evolutionary changes to account for the past and present diversity of morphologies and body plans. The genotype-phenotype relationships lie at the heart of evo-devo, which seeks to encompass in evolutionary theory what has been the black box of the genocentric neo-Darwinian 'Synthetic Theory'. This rapidly growing research program is undergoing what many consider to be a revolution in evolutionary theories. However, some authors cautiously point out that *"evo-devo hasn't quite lived up to expectations – at least, not if we were expecting a revolution in biology"* (Richardson, 2003, p. 351). At the same time, quoting Minelli (2003, p. 24) who rationally stressed that *"the role of genes in morphogenesis is likely always to be an indirect one"* Richardson observes that *"this is probably going too far for most of us, however"*. In these quotations lies the core of the issue regarding the gene centered view of development, which dominates many thoughts in evo-devo and relegates morphogenesis to what Fraser & Harland (2000) portray as *'the next frontier'* of biology. This is not in any way meant to dismiss research in developmental genetics or molecular biology. There is no doubt about the importance of the insights they provide. What should be pointed out, however, is that some authors, especially those engaged in the field of pattern formation and morphogenesis have repeatedly expressed their disappointment with this gene centered view. In regards to the inherently non-linear developmental dynamics, some expected revelations of evo-devo about genotype-phenotype relationships can not be yielded without a shift to an appropriate theoretical framework. The daunting challenge facing developmental biologists is a general one, not specific to biology. As any complex dynamic system, developing organisms exhibit emergent systemic properties that arise from non-linear interactions among system's components, at the tissular, cellular and subcellular levels. For example, some well documented experimental studies (not to mention theoretical ones) show that generic physical processes are involved in development and morphogenesis of such 'non-

anecdotic' features as neural tube (Steinberg, 1998) among other examples (e.g. Newman & Comper, 1990; Drasdo & Forgacs, 2000; Newman & Müller, 2000). However, the program metaphors, be they genetic or developmental, or related notions such as genetic instructions, persist in the works of some 'evo-devoists'. There is in fact no credible reason to claim such a genetic determinism of complex phenotypic traits. Context dependent and generic determinants of development and morphogenesis have nothing to do with a program or any metaphor implying a centralized controller. As Nijhout (1990, p. 443) pointed out, *"the network or pattern of gene activation does not constitute a program, it is both the consequence of, and contributor to, development…the only reasons for supposing the existence of a program for development are first, that we would have designed such a system that way, and second, that it is discomforting to deal with the notion that development is largely self-organizing. The main difficulty in accepting development as a self-organizing process is that we do not have a simple description of heritability and self-replication for such a system"*. Ten years later, Gilbert & Sarkar (2000, p.8) quite unfortunately note that *"one would have to look very carefully in any of the major developmental biology journals to find a differential equation or any other type of quantitative analysis. We have been having so much fun and getting so much data from our new molecular tools that we are prone to overlook new approaches that may enable us to solve important questions of differentiation and morphogenesis"*. This conceptual challenge demands a shift in the way causality is usually described. That development is a self-organizing process stresses that what can be defined as an underlying developmental process should not be sought for at the gene or gene product level, regardless of the level of organization at which the phenomenon of interest is observed. As argued by Strohman (1997), biology may be undergoing a Kuhnian revolution, one in which the existing genetic determinism will give way to a new conceptual understanding of the complexity of living organisms. As forcefully stressed by Huang (2000), post-genomic biology should not only deal with the dynamics of molecular networks, but also with the laws of macroscopic mechanics, especially in light of recent progresses in understanding the crucial role of physical forces (such as tension, compression, or shear stress) in switching cells between distinct fates (growth, differentiation or apoptosis) (e.g. Huang & Ingber, 1999). Strohman (2002, p. 701) captured the core of the issue in stressing that *"molecular biologists have rediscovered the profound complexity of the genotype-phenotype relationship, but are unable to explain it"*. Of course, the challenge defies all superlatives and requires an interdisciplinary approach with biological, mathematical, and physical inputs. However, this revealed complexity and inherently non-linear developmental dynamics, that do not fit with the linear way of thinking of the so-called central dogma of molecular biology, would have to lead 'evo-devoists' to purge their explanatory mode of outdated metaphors that so much helped to put development into a black box. Most 'evo-devoists' are undoubtedly well aware that such metaphors are no more than a convenient picture that should not be taken literally. What we wish to point out here, is that in keeping on making use of convenient but outdated metaphors, some 'evo-devoists' are not doing evo-devo any good in paving the way for other workers that persist to employ such 'instructionist' notions in a more literal and misleading perspective. It is beyond the scope of this paper to enter this controversy

fully. We will only illustrate this counter productive danger by critically reviewing two recently published studies we believe to be paradigmatic of deficiencies of genetic determinism, once reductionism ceases to be merely methodological. Our purpose is to exemplify how the persisting unclear role of genes in morphogenesis can help to keep the role of development marginalized, as well as to deny concepts lying at the very core of evo-devo.

II. A putative re-evolution of shell coiling

In a recent review, Pagel (2004) discusses a molecular phylogeny performed by Collin & Cipriani (2003) suggesting that shell coiling has re-evolved at least once in a family of limpets (Calyptraeidae, Gastropoda). Among systematists, it is mainly assumed that 'coiled' shells are ancestral, the limpets being considered as derived from coiled shells and undergoing secondarily uncoiling. Moreover, limpets are usually viewed as evolutionary dead ends, incapable to give rise again to the diversity seen among coiled shells (McLean, 1981). But the new molecular phylogeny of Collin & Cipriani suggests that the coiled shell genus *Trochita* (Schumacher, 1817) could be a derived form, firmly rooted within a clade of *Crepidula* (Lamarck, 1799) species. As *Crepidula* species are treated as uncoiled by the authors (but see our critics below), the ancestor of both *Crepidula* and *Trochita* is unambiguously reconstructed as being uncoiled. Thus *Trochita* is supposed to have re-evolved its coiled shell from an uncoiled ancestor. This new phylogeny is interpreted as a violation of Dollo's law – *"the irreversibility of evolution"*- understood as the implausibility or impossibility for complex ancestral states, once lost, to be regained in an evolutionary lineage. According to Collin & Cipriani (2003), shell coiling in *Trochita* has been reacquired by heterochrony, or as formulated by Pagel (2004), *"by the mechanism of prolonging the period during which genes for coiling are expressed in larvae"*. Then the permanent expression of these *'genes for coiling'* in larvae is supposed to explain the maintenance of the genetic and developmental integrity underlying coiling during 20-100 millions years of secondarily uncoiled evolution. Although we do not dispute the authors' phylogenetic hypothesis, we will stress that this putative re-evolution relies on an arbitrary scoring procedure of shell coiling, which can only be made 'intelligible' within a genocentric view. More generally, we will argue that the way in which the authors connect this case study to evolutionary theories stems from the unwarranted premise of a linear mapping of genes onto phenotypes where particulate inheritance of morphological characters seems implicitly assumed. We examine the underlying and often implicit assumptions associated with their interpretation. We concentrate on Pagel's review although some of our comments also apply to the original paper of Collin & Cipriani.

III. Is there 'genes for' coiling?

In our view, the gastropod shell and its coiling are undoubtedly complex characters, but obviously not in the sense of Collin & Cipriani and Pagel, who anyway do not give any definition of this term, but obviously rely on the mistaken 'complex-equals-genetic' premise. Like any other trait, shell form emerges as a developmental outcome of interactions between the component elements of a non-linear system, at the molecular, cellular and tissular levels. Treating biological form as a developmental outcome of processes hidden in the black box of some 'genes for' is an evasion

that leads to direct attention away from morphogenetic studies. How this view of development could account for mechanical phenomenon such as shear, folding and buckling, which are known to be involved in morphogenesis? How could a gene centered view (specific genes for specific coiling parameters) account for the fact that in gastropods the previous whorl acts as a template in partly determining shell coiling as suggested by Hutchinson (1989) on the basis of theoretical studies, and experimentally verified (Checa, Jimenez-Jimenez & Rivas, 1998)? How could this view integrate the role of mechanical forces at work in shell morphogenesis (see Morita, 1991a; 1991b)? These well documented studies may appear fairly 'simple' but are already far more complex, non-linear, than the naive idea that ultimate control of shell coiling resides in the genes viewed as encapsulated units of heredity. For example, generic physical properties of cells and tissues that influence morphogenesis may be thought as partly determined by the distribution of protein complexes and ultimately by genes encoding these proteins. But epigenetic processes imply a level of complexity beyond gene-gene interactions. Genes are involved in the phenotypic outcome of developmental dynamic, but the manifold determinants of morphogenesis lie at higher levels of organization including the level of the cell, the level of cell-cell networking, the tissular level and its generic physical properties, and the developing organism as a whole. All these levels have their own emergent rules, they interact one with another and with the environment and are not reducibly connected. These multilevel epigenetic interactions reduce our ability to draw the limits of network interactions involved in developmental dynamics and morphogenesis. They show at least that shell coiling cannot be esoterically explained as the product of pre-coded instructions hardwired in hypothetical 'genes for'. Genes do not function like an architect's blueprint and should not be thought of as specifying form. In other words, to postulate 'genes for coiling' as ultimate control for phenotypic expression does not only explain anything about snail development and evolution, but more critically gives spurious answer without having to bridge the gap between the levels of organization of the primary structure of proteins and the generation of three dimensional macroscopic form.

IV. Is *Crepidula* uncoiled?

In the foreword of the Wagner's (2001) edited volume on the character concept, Lewontin (2001, p. XVII) laid out a recurring question which pervades all areas of evolutionary biology: *"how are we to recognize the 'true' characters of organisms rather than imposing upon them arbitrary divisions that obscure the very processes that we seek to understand?"*. Undoubtedly, the most questionable point relates to the morphological interpretation Pagel and Collin & Cipriani have made. Anyone having seen a *Crepidula* should be surprised by Pagel's claim that *"the new phylogenetic position of Trochita is striking in that none of the Crepidula has a coiled shell"*. Pagel explains that *"to be designated as coiled a species had to return scores on these measures that were similar to a known coiled shell type"*. This procedure leads to consider as uncoiled every shell which is less coiled than a chosen reference. Applied to snail shells, this procedure may have appeared rational at first glance. But just imagine what it could involve if applied to other quantitative characters such as the length of tetrapod limbs: could shorter limbs really be coded as limblessness?! The

shell coiling morphospace provided by Collin & Cipriani even shows that *Crepidula* appears as coiled as the shell reference. As this quantification of shell coiling does not equate with the unsubstantiated presupposition that *Crepidula* is uncoiled, Collin & Cipriani finally *"coded species with shells that did not complete a single rotation as uncoiled in the subsequent analysis"*. This premise cannot be rationally justified, especially within the framework of this subject. Does it mean that, when applied to other snail species, this procedure would have the implication that as many 'genes for' are required for successive whorls or that the first whorl requires specific genes? Does a snail with 4 whorls may be considered more complex than another one with only 3 whorls? Is there any discrete change in shell morphology between 0,9 and 1,1 whorl that could be linked to major modifications in developmental dynamics? *Crepidula*, like many other gastropods that normally clamp onto rock surfaces or other shells, exhibit a great amount of phenotypic polymorphism and morphological plasticity. Coiling parameters are notoriously variable. Within the same population of *C. fornicata* (Linnaeus, 1758), for example, some adult specimens do complete a single whorl or do not (Fig. 1). But even these last specimens cannot be considered as uncoiled. It has been recognized for more than a century that mollusks shells share the same basic spiral geometry, regardless of the number of whorls (Moseley, 1838; D'Arcy Thompson, 1952; Raup, 1966). In spite of often not completing a single whorl, limpets such as *Crepidula* are spirally coiled. Likewise, all prosobranch larvae are coiled, contrary to

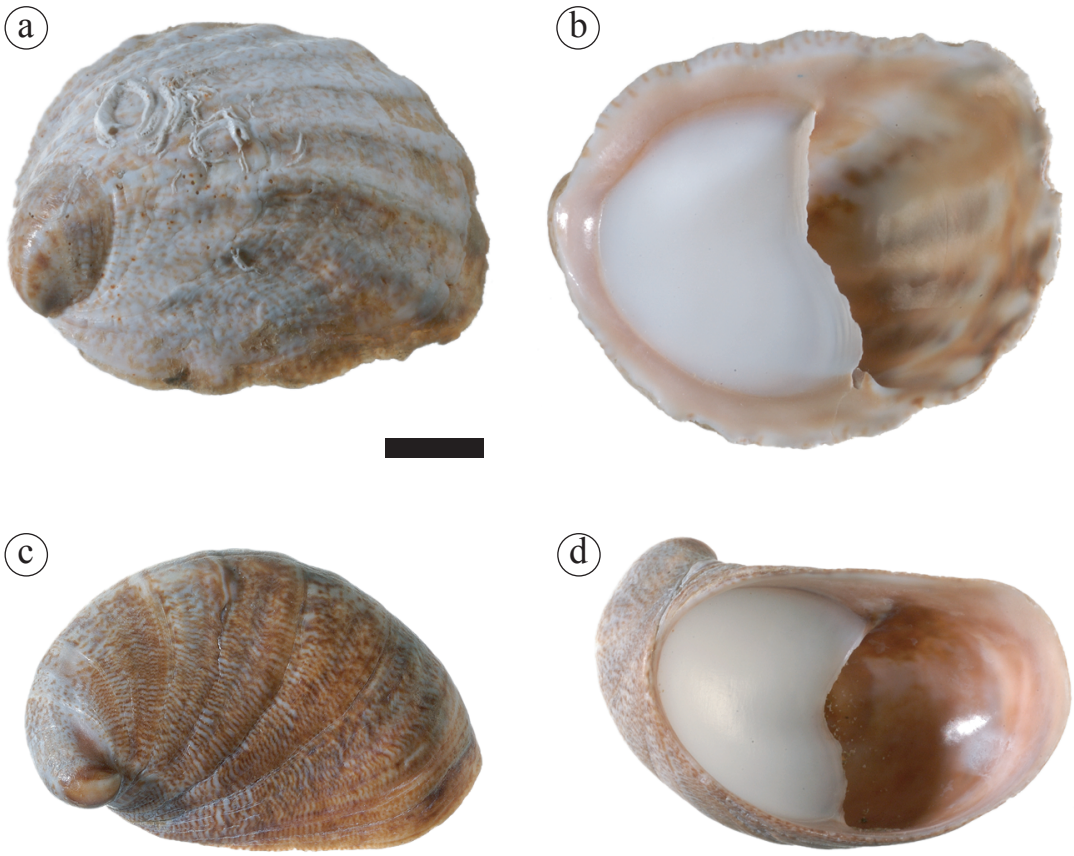


Figure 1: **a-d**. Ecophenotypic intrapopulational variation in *Crepidula fornicata* (Linnaeus 1758) (Arromanches, France). **a-b**. A specimen with less than one whorl, and unusual ribs matching the relief of the flat left valve of a Pecten onto which it was clamped. **c-d**. A specimen with more than one whorl (Scale bar: 10 mm).

what seems to be assumed by Collin & Cipriani and other taxonomists as well. One can perhaps qualify such shells of 'uncoiled' (i.e. Ponder & Lindberg, 1997 among others) for descriptive purposes, however when it comes to identifying characters, one has to wonder on which grounds two 'apparently' different shapes can be divided into discrete units. Coiling around an axis implies some ineluctable rules of geometry, the most evident being that for the same apertural growth rate, a loosely coiled shell must grow for a much longer time than a tightly coiled one to complete a single whorl. Then, the fact that some specimens complete or not a single whorl (or more) cannot be considered as discrete character for which the application of maximum likelihood reconstruction of ancestral states (Pagel, 1999) could be rationally justified, as Collin & Cipriani assume.

V. Does heterochrony explain the re-evolution of a previously lost character?

Since Collin & Cipriani suppose that coiling has 're-evolved' by heterochrony, one might wonder if such a phylogenetic study could really be considered as a compelling example of recurrence of a previously lost complex character. After all, a shift in developmental timing of a character means that this character has not been lost during evolution. From this point of view, it is also worth noting caution expressed some 15 years ago by Raff & Wray (1989) who pointed out that the concept of heterochrony, if taken in a too wide sense, could apply to every change in evolutionary history. Furthermore, if any change in shell coiling parameters is relevant to discuss the issue of recurrence of a previously lost complex character, undoubtedly countless examples of violation of Dollo's law (but see below) may be found in the evolution of snails and other mollusks. The highly homoplastic character of shell coiling remains a major wisdom among malacologists. As argued by Rice (1998), a loosely coiled shell could theoretically be derived from a tightly coiled shell by reducing the total amount of shell produced. Could the reverse really be impossible? Then, 're-evolution' of a tightly coiled shell from a loosely coiled one could only result from the changing of some growth parameters. It does not necessarily imply that some developmental pathways may be lost and then re-activated. Unfortunately, some authors rely on this assumption to argue that re-evolution is unlikely.

VI. What about Dollo's law?

In 1893, Dollo (p. 165) gave the following formulation: *"An organism cannot come back, **even partially**, to a previous state, which has already been realized in the series of its ancestors"* (emphasis added). Later, however, some critics have led Dollo (1905, p. 443) to reformulate his law in this way: *"An organism never comes back **exactly** to its previous state due to the indestructible nature of the past, it always retains some trace of the transitional stages through which it has passed"* (emphasis added). Although numerous examples may probably violate the first law (depending on the view of what 'partially' means, as exemplified by the interpretation of shell coiling), no example can be viewed as a violation of the rather pointless second law, which can anyway be applied to any historical science. Moreover, it should be noted that contrary to what had been assumed by many authors, Dollo did not use the argument of complexity in his first or second formulation, nor he had restricted his law to the case of previously lost characters.

Dollo's law addresses only the idea of irreversibility in evolution and historical contingencies, which can be seen as the most simple and quite pointless definition of phylogenetic constraint.

VII. The maintenance of the adaptationist program

Pagel (2004) states that the view according to which *"Nature is limited in what it can produce, being constrained by a few archetypes or plans, remains influential even today, finding life in phrases such as 'phylogenetic constraint' and 'phylogenetic inertia'. But it is a view with little supporting empirical or theoretical evidence"*. It should be reminded that when Gould & Lewontin (1979) coined the term 'phyletic constraint', it was to challenge the neo-Darwinian tendency to disregard history within the framework of adaptationist interpretations and its related principle of an all-powerful natural selection. However, without premises as to what kind of evolutionary process Pagel encompasses under the umbrella of 'Nature', his statement remains difficult to debate. Does it mean that natural selection is unlimited in what it can achieve from some starting point? Obviously, considerable evolution goes on within body plans and as Raff (1996, p. 180) pointed out, *"a Tyrannosaurus doesn't much resemble an Amphioxus or a primitive jawless vertebrate like a lamprey"*. The most critical issue to test historical limitations relates to the specification of an appropriate null hypothesis in comparative studies, what Pagel misses to do in order to assert his 'unconstrained' view. For more than twenty years, many authors have acknowledged, however, that the heuristic value of the concept of constraint does not lie in the idea of limitation since one can say for certain that any mechanism in our living and physical world must have some limits. Moreover, the role of natural selection is inherently limited, in that it can only influence the distribution of existing phenotypes and does not account for the origin of variation. In fact, the interest in constraints, especially developmental constraints, rather resides in the notion of production of phenotypic variation biased by the dynamics of developmental systems (e.g. see Fusco, 2001; Arthur, 2002 for reviews). This concept relates to the non-linearity of the mapping from genes to phenotypes and the emergent properties of developmental systems at each level of organization biasing the pattern of heritable phenotypic variation. The fundamental notion, which is at the very core of evo-devo, is that *"in evolution, selection may decide the winner of a given game but development non-randomly defines the players"* (Alberch, 1980; p. 665). In other words, even though genetic mutations are random, some degree of phenotypic order (e.g. non-random variation of traits, correlation among them...) may be due to developmental bias related to a common set of generative processes. They are essential to understand how a trait can be produced during ontogeny and modified through phylogeny. Thus, the effects of developmental constraints must be discussed along with the phylogenetic ones, because the two areas are closely intertwined, and in some cases, evidence for the former may provide converging evidence for the later. This is remarkably illustrated by many studies on tetrapod limbs (e.g. Oster *et al.*, 1988). Likewise, numerous studies emphasized that mollusks shell shapes underlie common generative processes, their most basic expression being the logarithmic spiral (or nearly so). Should we see in this feature and its associated universal properties the unexpected result of historical contingencies, selected at random from an unlimited range of

others? Without doubt, many *ad hoc* hypotheses may be introduced to privilege the idea that *"apparent conservatism in some traits could say more about their exceptional adaptive value than about any inability to alter them"* as Pagel suggests. However, it remains unclear what kind of 'exceptional adaptive value' could account for shell coiling in mollusks from the Cambrian to present, within various marine, freshwater, and terrestrial environments.

Quite unsurprisingly, Pagel claims that *"studies such as Collin and Cipriani's paint a picture of Nature not as a mere tinkerer but as an architect capable of producing designs for the particular place and time, even if it means reusing very old plans"* but he does not seem to see in this 'reusing' any evidence of historical limitations. This teleological statement amounts to nothing more than to disregard that developmental genetics has given increasing support to Jacob's metaphor (1977) in revealing the wide-ranging recruitment of regulatory networks among metazoans (although it should be noted that this metaphor was coined in a selectionist perspective). Moreover, this view is reminiscent of the classical 'argument from design' that pervades neo-Darwinian paradigm. In its theological formulation, the logic of the argument from design is that any complex structure implies the existence of a purposeful 'designer', Paley's 'watchmaker'. In its neo-Darwinian version, the argument from design stems from the recognition of functional characters and the inference that these traits have been built by natural selection, Dawkins' (1986) 'blind watchmaker'. The complexity of design is a key argument and is held to provide evidence of action of natural selection (see Lauder, 1996). This program has lead to reduce biological form to biological function and then biological function to natural selection. This basic premise has a teleological component specifying what phenotype should be to achieve a prescribed end. Thus, teleological language is commonly justified by reference to natural selection and recourse to man-made device, engineer or architect analogies. The fact that Pagel invites us to *"just compare the mobility of the robotic Martian rovers currently inching around the surface of that planet to that of just about any tetrapod"*, in support of his argument about the couple **'**conservatism-exceptional adaptive' value, is in the straight line of the classical argument from design. Most evolutionists are well aware that we are not bound to take such analogies seriously in view of the misleading imagery they evoke about phenotypic evolution. The main difference between biological and technological evolution does not reside in the functional aspects of the systems, but in their construction. A man made device exists because it has been designed and built to perform a given function, determined from the outside by a plan that contains all the complexity of the end product. It is not the case for self-organized processes that characterize the development of the biological form.

To presuppose that selection builds traits in the same way as an engineer (or an architect) would design a piece of a machine to perform a task underlies an atomistic philosophy. From this point of view, it is worth recalling the perspective in which emerged the concept of developmental constraint: within the neo-Darwinian framework, natural selection is not only a filter of phenotypic variation, but it also builds it. Since the basic assumption is that genotype determines phenotype in all respects, a fine-tuning process of natural selection assumes the role of a 'designer' in incrementally building a new phenotype out of the

randomly generated raw genetic material. An implication of this view is the widespread recourse to the metaphor of genetic program[1], coined by Jacob & Monod (1961, p. 354) in their paper on the operon model for gene regulation, where they state *"that the genome contains not only a series of blueprints, but a coordinated program of protein synthesis and the means of controlling its execution"*. This metaphor has been quickly extended by Mayr (1961, p. 1503-1504), who tell us that *"an individual who—to use the language of the computer—has been 'programmed' can act purposefully..... Natural selection does its best to favor the production of codes guaranteeing behavior that increases fitness.... The purposive action of an individual, insofar as it is based on the properties of its genetic code, therefore is no more no less purposive than the actions of a computer that has been programmed to respond appropriately to various inputs"*. Such deterministic and teleological views have had many unfortunate consequences, the most basic being that development was eclipsed altogether. The belief that DNA produces organisms out of a linear computer-like program has lead to the illusion that no developmental explanation is needed for traits that are hardwired in the genes. *Reductio ad absurdum*: this teleological picture leads to that offered by Dawkins (1976, p. 71), in which *"natural selection favours genes which control their survival machines in such a way that they make the best use of their environment"*. What is important for our purpose is that genetic determinism along with the perceived complexity of 'design' is at the core of Pagel's argumentation. An assumption should be made explicit in Pagel's view: reversibility presupposes that phenotypes may be regarded as decomposable aggregates of stable units that can be taken apart again, at the right place and time, awaiting activation by some implicit functional demand. Pagel relies on the belief that a quantitative trait such as shell coiling is tractable in terms of specific genes in privileging the idea, without factual argument, that this putative re-evolution implies the same ancestral *'genes for coiling'*. Such assumptions can only survive within a gene centered view. This claim rests on the assumption that genes carry a set of pre-specified instructions for phenotypic traits and that one may justifiably give a meaningful explanation of anything thanks to the *ad hoc* rescue of abstract 'genes for', the innermost locus at which we should seek an answer about development and evolution. In other words, the very absence of knowledge of what these 'genes for' are, makes it easier to attribute them any properties. Moreover, to encapsulate an abstract collection of genes into an autonomic entity has an operational purpose: the view that inherited phenotypic change is just a matter of inheritance of specific genes remains a purposeful fiction of the adaptationist program, in keeping tractable atomistic phenotypic selectionism. From this point of view, it is worth noting that the notion that phenotypic traits can be individually 'targeted' by natural selection is at the heart of some criticisms (Leroi, Rose & Lauder, 1994) against the phylogenetic comparative methods to the study of adaptation (Harvey & Pagel, 1991).

VIII. Conclusion

We do not dispute the possibility of recurrence of a complex character previously lost during evolution, although it should be stressed that without qualification as to what 'complex trait' means,

1 Although the 'genetic program' metaphor has been first introduced by Schrödinger (1944) in his book '*What Is Life?: the physical aspect of the living cell. Cambridge University Press, Cambridge.*' the paper by Jacob & Monod (1961) can be viewed as the first influential use of this metaphor in biology, especially because it relied on a model of gene regulation.

the question of the frequency of this evolutionary phenomenon will remain without compelling answer. Pagel quotes as an example a recently published phylogenetic study suggesting that wings may have reappeared several times within the ancestrally wingless stick insects (Whiting & Whiting, 2003; but see Trueman *et al.,* 2004). But he overlooks the fact that in this example, the characters are discrete and do not need artificial scoring to be studied. This is obviously not the case for shell coiling.

IX. References

Alberch, P. (1980). Ontogenesis and morphological diversification. *American Zoologist* **20,** 653-667.

Arthur, W. (2002). The emerging conceptual framework of evolutionary developmental biology. *Nature* **415,** 757-764.

Checa, A. G., Jimenez-Jimenez, A. P. & Rivas, P. (1998). Regulation of spiral coiling in the terrestrial gastropod Sphincterochila: an experimental test of the road-holding model. *Journal of Morphology* **235,** 249-257.

Collin, R. & Cipriani, R. (2003). Dollo's law and the re-evolution of shell coiling. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270,** 2551-2555.

Dawkins, R. (1976). *The Selfish gene*. Oxford University Press, Oxford.

Dawkins, R. (1986). *The Blind Watchmaker*. Norton, New York.

Dollo, L. (1893). Les lois de l'évolution. *Bulletin de la société belge de géologie, paléontologie et hydrologie* **7,** 164-166.

Dollo, L. (1905). Les dinosauriens adaptés à la vie quadrupède secondaire. *Bulletin de la societé belge de géologie, paléontologie et hydrologie* **19,** 441-448.

Drasdo, D. & Forgacs, G. (2000). Modeling the interplay of generic and genetic mechanisms in cleavage, blastulation, and gastrulation. *Developmental Dynamics* **219,** 182-191.

Fraser, S. E. & Harland, R. M. (2000). The molecular metamorphosis of experimental embryology. *Cell* **100,** 41-55.

Fusco, G. (2001). How many processes are responsible for phenotypic evolution? *Evolution & Development* **3,** 279-286.

Gilbert, S. F. & Sarkar, S. (2000). Embracing complexity: organicism for the 21st century. *Developmental Dynamics* **219,** 1-9.

Gould, S. J. & Lewontin, R. C. (1979). Spandrels of San-Marco and the panglossian paradigm: a critique of the adaptationist program. *Proceedings of the Royal Society of London Series B-Biological Sciences* **205,** 581-598.

Harvey, P. H. & Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Huang, S. (2000). The practical problems of post-genomic biology. *Nature Biotechnology* **18,** 471-472.

Hutchinson, J. M. C. (1989). Control of gastropod shell shape: the role of the preceding whorl. *Journal of Theoretical Biology* **140,** 431-444.

Jacob, F. (1977). Evolution and tinkering. *Science* **196,** 1161-1166.

Lauder, G. V. (1996). The argument from design. In *Adaptation* (ed. M. R. Rose and G. V. Lauder). Academic Press, San Diego.

Leroi, A. M., Rose, M. R. & Lauder, G. V. (1994). What does the comparative method reveal about adaptation? *American Naturalist* **143,** 381-402.

Lewontin, R. C. (2001). Foreword. In *The Character Concept in Evolutionary Biology* (ed. G. P. Wagner), pp. XVII-XXIII. Academic Press, San Diego.

Mayr, E. (1961). Cause and effect in biology. *Science* **134,** 1501-1506.

McLean, J. H. (1981). The Galapagos rift limpet *Neomphalus*: relevance to understanding the evolution of a major paleozoic-mesozoic radiation. *Malacologia* **21,** 291-336.

Minelli, A. (2003). *The Development of Animal Form: Ontogeny, Morphology, and Evolution*. Cambridge University Press, Cambridge.

Morita, R. (1991a). Finite element analysis of a double membrane tube (DMS-tube) and its implication for gastropod shell morphology. *Journal of Morphology* **207,** 81-92.

Morita, R. (1991b). Mechanical constraints on aperture form in gastropods. *Journal of Morphology* **207,** 93-102.

Moseley, H. (1838). On the geometrical forms of turbinated and discoid shells. *Philosophical Transactions of the Royal Society of London* **128,** 351-370.

Newman, S. A. & Comper, W. D. (1990). "Generic" physical mechanisms of morphogenesis and pattern formation. *Development* **110,** 1-18.

Newman, S. A. & Muller, G. B. (2000). Epigenetic mechanisms of character origination. *Journal of Experimental Zoology* **288,** 304-317.

Nijhout, H. F. (1990). Metaphors and the role of genes in development. *BioEssays* **12,** 441-446.

Oster, G. F., Shubin, N., Murray, J. D. & Alberch, P. (1988). Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. *Evolution* **42,** 862-884.

Pagel, M. (1999). The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* **48,** 612-622.

Pagel, M. (2004). Limpets break Dollo's law. *Trends in Ecology & Evolution* **19,** 278-280.

Ponder, W. F. & Lindberg, D. R. (1997). Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. *Zoological Journal of the Linnean Society* **119,** 83-265.

Raff, R. A. (1996). *The shape of life: Genes, Development and the Evolution of Animal Form*. University of Chicago Press, Chicago.

Raff, R. A. & Wray, G. A. (1989). Heterochrony: developmental mechanisms and evolutionary results. *Journal of Evolutionary Biology* **2,** 409-434.

Raup, D. M. (1966). Geometric analysis of shell coiling: some general problems. *Journal of Paleontology* **40,** 1178-1190.

Rice, S. H. (1998). The bio-geometry of mollusc shells. *Paleobiology* **24,** 133-149.

Richardson, M. (2003). A naturalist's evo-devo. *Nature genetics* **34,** 351.

Steinberg, M. S. (1998). Goal-directedness in embryonic development. *Integrative Biology* **1,** 49-59.

Strohman, R. (2002). Maneuvering in the complex path from genotype to phenotype. *Science* **296,** 701-703.

Strohman, R. C. (1997). The coming Kuhnian revolution in biology. *Nature Biotechnology* **15,** 194-200.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Trueman, J. W. H., Pfeil, B. E., Kelchner, S. A. & Yeates, D. K. (2004). Did stick insects *really* regain their wings? *Systematic Entomology* **29,** 138-139.

Wagner, G. P. (2001). *The Character Concept in Evolutionary Biology*. Academic Press, San Diego.

Whiting, M. F. & Whiting, A. S. (2004). Is wing recurrence *really* impossible?: a reply to Trueman *et al*. *Systematic Entomology* **29,** 140-141.

"Our truth is the intersection of different lies".

Levins, 1966, p. 423.

Part II:

Molluscan shell shape:

growth models and patterns of variation

General introduction to the models of molluscan shell morphogenesis

Contents

In the previous part of this dissertation, it was pointed out that biological organization could be viewed as generic, sometimes quasi-universal properties of matter. The formation of biological shapes is a fascinating area of scientific research, especially because it is generally sufficient to hypothesize simple rules to generate complicated outcomes with a 'realistic' look.

Different categories of models have been applied to the morphogenesis of the molluscan shells. These models can roughly be divided into three groups: those dealing with pattern formation and chemical organization at a molecular/cellular level, those concerned with mechanical aspects and focusing on the cellular/tissue level and those dealing with form and growth description at the organism level. The latter kind of models is used in this dissertation and widely discussed in chapters 3 & 4 and to a lesser extent in chapter 5. The two other kinds of models, reaction-diffusion and mechanical models are briefly discussed below with respect to their application to molluscan shell morphogenesis (see chapter 1 for more examples and extensive discussion).

I. Lateral inhibition models

Pattern formation is strictly defined as the dynamical process by which spatio-temporal arrangements of repeated features arise. The reaction-diffusion models root in the pioneer work of Turing (1952) who first proposed that a set of interacting and diffusing activator-inhibitor molecules could destabilize itself to lead to an inhomogeneous distribution of the concentration of these molecules in the developing field (diffusion-driven instability). These models highlighted the emerging capabilities of molecular networks through local self-enhancement and lateral inhibition. The signalling molecules, the morphogens, have then been attributed the role of controlling the subsequent differentiation of the cells during morphogenesis. The spatio-temporal distribution of these morphogens is often named "pre-pattern". This implies the idea that the emerging distribution of the gene products will control the differentiation of the cells to finally create the associated pattern. Reaction-diffusion models have been largely developed by Meinhardt and his coworkers (e.g. Meinhardt

& Gierer, 1974) and applied to the pigmentation of sea shells to reproduce a vast diversity of coloration patterns (Meinhardt & Klinger, 1988; Meinhardt, 1995).

Another type of lateral inhibition model is the neuronal model of Ermentrout, Campbell & Oster (1986) based on cellular automata. This model is used to simulate pigmentation of sea shells where positive and negative influxes of neurons are computed. These influxes are then supposed to trigger or prevent the secretion of pigment by the mantle cells.

Several authors proposed models based on biochemical reactions or neural networks to account for the formation of ornamentation or shell growth (Meinhardt, 1995; Savazzi, 1990; Hammer & Bucher, 1999; Guex *et al*., 2003; Hammer & Bucher, 2005).

However, as pointed out in chapter 1, a general drawback of reaction-diffusion models is the difficulty of linking these microscopic descriptions of processes (which echo the genetic level of description of development) to the three-dimensional macroscopic shape changes that have to be explained, especially when biochemical components, cell movements and tissues continuously interact to generate structures of specific shape (Murray, 1989). Goodwin (1988, p. 636) captures the core of the issue by saying that "*Turing's achievement was remarkable, but it does not provide the solution to the problem of morphogenesis. The reason is that a spatial pattern in the concentration of metabolites within a developing organism does not itself explain the actual geometry of say, the tentacles on a hydroid, the leaves on a plant, or the limbs of an amphibian. Morphogenesis, as the name implies, is the generation of structures of specific shape, whereas spatial patterns of chemical concentration arise within some pre-defined geometry. In order to get morphology, work has to be done in deforming cells or cell sheets into specific shapes, and growth must be localized to generate specific structures*". This issue can be overcome if one takes conjointly into account the mechanical aspects of morphogenesis (e.g. Oster *et al.*, 1988) or simulates some kind of feedback between the interacting biochemical components (genes products) and the growth of the developing structure, an approach that has been called 'morphodynamic' in opposition to pure reaction-diffusion models that are qualified as 'morphostatic' (Salazar-Ciudad, Jernvall & Newman, 2003).

II. Mechanical models

Other types of models do not rely on regulatory networks, but rather deal with the mechanical aspects of shell growth. Morita (1991a) developed a model focusing on the mechanics of the mantle. Here, the mantle is simulated as a double elastic membrane and its physical state is supposed to be in balance between its internal stress and the forces acting on it (e.g. pressure of the haemolymph, boundary of the shell, pressure induced by the foot). The deformation of the mantle is then deduced from its stress field. This kind of model includes several aspects on the subsequent deformation of the mantle such as the influence of the whorl overlap or the effect of the previously secreted shell (Hutchinson, 1989). In that way, this model suggested several constraints upon the shell morphology of gastropods explaining how the coiling direction and the aperture shape could be determined (Morita, 1991b). This author suggested that the muscle patterns and modes of coiling were developmentally coupled so that malfunctioning combinations would be excluded without natural selection. This model

raises the question how the growth process of the mantle could be related to its mechanical state which is dependent upon genes, mode of life, growth environment and the previously secreted shell segments (Morita, 1993; 2003).

Another type of model developed by Hammer (2000) suggests that a regulative feedback system with delay based on purely mechanical detection can control apertural growth rate. This assumption appears sufficient to explain the occurrence of commarginally ribbed shells (ornamentation parallel to the aperture).

III. Form and growth models

The third kind of models, dealing with the description of shell form and growth has a long history, since simple geometric consideration about logarithmic spirals date back to Moseley (1838). The contribution of D'Arcy Thompson (1952) to the problem of molluscan shell form is also notorious. Raup's work (1961) significantly contributed to the increasing interest in the simulation of shells with the help of computers. Since then, geometrical models progressively shifted from shape description to growth description, while more and more realistic assumptions were taken (e.g. no coiling axis, discrete growth, consideration of timing). These models and their assumptions will be discussed in chapter 3.

IV. Which model for which purpose?

There exist a wide range of models for molluscan shell morphogenesis, from shape models to mechano-chemical models. Relating shell shape to genetic and environmental parameters would obviously require the investigation of the mechano-chemical aspects of shell morphogenesis which provide the 'causal' factors determining the shape and size of growth increments at each growth step upon the constraints of previous built shell. Such analyses are notoriously challenging, especially because of the non-linearity of soft tissue growth and genes interactions, and perhaps above all because most of molluscan development is unknown.

A higher level of description can be shown to be a suitable alternative to investigate the relationships between growth and form, an approach particularly encouraged by the simple morphology of molluscan shells. This approach favours the comparison between theoretically and empirically derived patterns of shape variation, a point essential to this dissertation. As illustrated in chapter 5, the understanding of the relationships between growth and shape variation is challenging. Shell shape variation can hardly be interpreted at all in the absence of a null hypothesis model dealing with the relationships between growth and shape. Thesecond part of this dissertation is a contribution to the following questions:

(1) What kind of morphological variation is expected given some basic rules of growth?

(2) What kind of rules could underlie the observed patterns of variation?

Chapters 3 & 4 explore the first question and highlight the usefulness of null hypothesis models. **Chapter 3** has implications regarding the (much forgotten) relationship between growth rates and allometry in general and molluscs in particular. **Chapter 4** highlights how different sub-data sets could lead to different interpretations of the same phenomenon at the level of populations. It also points out to the impossibility, both empirically but more importantly theoretically, to determine

how much variation is due to variation in 'genotype' and how much of it is due to the variation in the 'environment'. **Chapter 5** explores the second question, which is undoubtedly the more challenging. Besides describing the individual patterns of ontogenetic variation in shell growth and shell shape in a population of *Hexaplex trunculus,* the growth vector model, developed in chapters 3 & 4, is used to suggest what kind of rules could underlie these patterns. Then, from chapters 3 to 5, we will progressively move from patterns of ontogenetic variation to patterns of phenotypic variation in populations, while trying to keep the link between the two. These patterns are suggested to be a reflection of simple growth rules tied to accretionary growth. If so, it is expected that similar patterns of variation may be found in other molluscs, leading to convergent patterns of evolutionary transformations.

V. References

Ermentrout, B., Campbell, J. & Oster, G. (1986). A model for shell patterns based on neural activity. *Veliger* **28,** 369-388.

Goodwin, B. C. (1988). Problems and prospects in morphogenesis. *Experientia* **44,** 633-637.

Guex, J., Koch, A., O'Dogherty, L. & Bucher, H. (2003). A morphogenetic explanation of Buckman's law of covariation. *Bulletin de la Société Géologique de France* **174,** 603-606.

Hammer, O. (2000). A theory for the formation of commarginal ribs in mollusc shells by regulative oscillation. *Journal of Molluscan Studies* **66,** 383-391.

Hammer, O. & Bucher, H. (1999). Reaction-diffusion processes: application to the morphogenesis of ammonoid ornamentation. *Geobios* **32,** 841-852.

Hammer, O. & Bucher, H. (2005). Models for the morphogenesis of the molluscan shell. *Lethaia* **38,** 111-122.

Hutchinson, J. M. C. (1989). Control of gastropod shell shape: the role of the preceding whorl. *Journal of Theoretical Biology* **140,** 431-444.

Meinhardt, H. (1995). *The Algorithmic Beauty of Sea Shells*. Springer-Verlag, Berlin.

Meinhardt, H. & Gierer, A. (1974). Applications of a theory of biological pattern formation based on lateral inhibition. *Journal of Cell Science* **15,** 321-346.

Meinhardt, H. & Klingler, H. (1988). A model for pattern formation of shells of mollusks. *Journal of Theoretical Biology* **126,** 63-89.

Morita, R. (1991a). Finite element analysis of a double membrane tube (DMS-tube) and its implication for gastropod shell morphology. *Journal of Morphology* **207,** 81-92.

Morita, R. (1991b). Mechanical constraints on aperture form in gastropods. *Journal of Morphology* **207,** 93-102.

Morita, R. (1993). Development mechanics of retractor muscles and the "Dead Spiral Model" in gastropod shell morphogenesis. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **190,** 191-217.

Morita, R. (2003). Why do univalve shells of gastropods coil so tightly? A head-foot guidance model of shell growth and its implication on developmental constraints. In *Morphogenesis and Pattern Formation in Biological Systems: Experiments and Models* (ed. T. Sekimura, S. Noji, N. Ueno and P. K. Maini), pp. 345-354. Springer-Verlag, Tokyo.

Moseley, H. (1838). On the geometrical forms of turbinated and discoid shells. *Philosophical Transactions of the Royal Society of London* **128,** 351-370.

Murray, J. D. (1989). *Mathematical Biology*. Springer-Verlag, Berlin, Heidelberg.

Oster, G. F., Shubin, N., Murray, J. D. & Alberch, P. (1988). Evolution and morphogenetic rules: the shape of the vertebrate limb in ontogeny and phylogeny. *Evolution* **42,** 862-884.

Raup, D. M. (1961). The geometry of coiling in gastropods. *Proceedings of the National Academy of Sciences of the United States of America* **47,** 602-609.

Salazar-Ciudad, I., Jernvall, J. & Newman, S. A. (2003). Mechanisms of pattern formation in development and evolution. *Development* **130,** 2027-2037.

Savazzi, E. (1990). Biological aspects of theoretical shell morphology. *Lethaia* **23,** 195-212.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **237,** 37-72.

Chapter 3 - Allometries and the morphogenesis of the molluscan shell: a quantitative and theoretical model

Reference: Urdy, S., Goudemand, N., Bucher, H. & Chirat, R. Allometries and the morphogenesis of the molluscan shell: a quantitative and theoretical model. *Journal of Experimental Zoology Part B.* accepted.

Abstract

In this chapter, we examine the relationships between growth rate, shape and allometries of molluscan shells. After reviewing the previous theoretical approaches devoted to the understanding of shell form and its morphogenesis, we present a free-form vector model which can simulate apertural shape changes and non-linear allometries. In each simulation of a shell, the first growth increment defines so-called growth vectors which are assumed to be constant in direction (relative to the last computed aperture position). Shell morphology is generated by iteratively adding a growth increment onto the last computed aperture. Each growth increment is obtained by uniformly scaling the growth vectors according to various growth rate curves that are used to simulate the mantle growth over time. From the model, we derive morphometric variables that illustrate the ontogenetic trajectories in time-size-shape space. We investigate the effects of changing the growth curves types, growth rate parameters and growth vector maps on the direction, speed and patterns of ontogenetic allometries. This model illustrates some fundamental geometrical properties of the logarithmic spiral, in particular the close relationship between the size and the geometry of growth increments. More generally, this model highlights the role of growth rates in the generation of allometries. Moreover, this model can be used to develop a mathematically data-driven approach where experimentally obtained growth curves could be used as inputs in the model. Even if the model does not causally address the factors involved in shell growth and its secretion, it nevertheless paves the way towards the understanding of the mechano-chemical aspects of shell morphogenesis.

Key words: molluscs – growth – allometry – ontogeny – morphometry.

Contents

I. Introduction

At least since D'Arcy Thompson's time, it is recognized that differences in relative growth of body parts could account for much of the intraspecific variation of form, as well as a large part of the diversity found between taxa. D'Arcy Thompson's coordinate grid transformations clearly emphasized the necessity for analysing shape changes in their spatio-temporal dimensions since *"[i]n short it is obvious that the form of an organism is determined by its rate of growth in various directions…organic form itself is found, mathematically speaking, to be a function of time"* (Thompson, 1952, p.76).

Building on this spatio-temporal view of morphology, Alberch *et al.* (1979) coined the term 'ontogenetic trajectory' to describe the path followed by a particular organism ('s body part) during development through a multivariate space ('ontogenetic space'). They argued that ontogenetic trajectories should be best represented through an age-size-shape space. This attempt at devising a quantitative framework for describing the evolution of size and shape stimulated a wealth of studies which revealed important kinds of ontogenetic variation, interpreted in terms of heterochrony (e.g. Alberch *et al.*, 1979; Alberch & Gale, 1985; McKinney & McNamara, 1991), phenotype integration (e.g. Zelditch, 1988; Cheverud, 1982a) and allometry (e.g. Gould, 1966; Klingenberg & Froese, 1991). Before the 90's, morphometric tools were restricted to bivariate plots and multivariate statistics.

Since then, statistical methods have been extensively developed to apply the concept of D'Arcy Thompson coordinate grid transformations to the (semi-)quantitative study of morphology. Using thin-plate splines and multivariate statistics, the landmark based morphometric methods allowed the decomposition of biological shape changes into statistically independent directions of deformation (Bookstein, 1989; see review by Stone, 1997). The geometric morphometrics have the advantage of separating shape from size while maintaining the link between the derived shape variables (principal component scores) and the original landmark configurations (Bookstein, 1991; Rohlf & Slice, 1990; Sokal & Rohlf, 1995; Adams, Rohlf & Slice, 2004). Thus, in the multivariate size-shape space, ontogenetic trajectories can 'easily' be visualized. A wealth of studies has been carried out using such methods (see review by Roth & Mercer, 2000).

It turned out to be a challenge to use the empirical descriptions of allometry and heterochrony for drawing inferences about the underlying mechanisms. Over the past decades, the concept of heterochrony has been revisited many times, leading sometimes to contradictory interpretations when different frameworks were more or less implicitly endorsed (see Godfrey & Sutherland, 1995a, b; Klingenberg, 1998). In particular, it happened when studies based on the 'clock model' of Gould (1977) were compared to those based on the subsequent modification of this model by Alberch *et al.* (1979). Some ambiguity in definitions has been highlighted, especially with regards to the application of the concepts of heterochrony to molecular and cellular processes of morphogenesis (e.g. Raff & Wray, 1989; Alberch & Blanco, 1996). Thus, the transposition of a terminology traditionally belonging to comparative embryology and morphology to the cellular or molecular levels has turned out challenging for *"the temporal nature of development guarantees almost any change in developmental process will produce some effect on timing"* (Raff & Wray, 1989, p. 430). Ambiguity in definitions has resulted in a

situation whereby almost all morphological evolution has been attributed to heterochrony (e.g. see discussion by Webster & Zelditch 2005). As heterochronies at the morphological level may be heterogeneous or partially overlapping with regards to the changes at the genetic and/or cellular levels, many authors argue that the study of heterochrony at different levels requires separate treatment and terminology (e.g. Alberch & Blanco, 1996). It is also important to remind that the classical terminology of heterochrony is only adequate to describe changes in couples of ontogenetic trajectories as long as the same mathematical transformation can make them linear (Rice, 1997). If not, the changes from ancestor to descendant cannot be either meaningfully described in terms of the 6 'pure' types of heterochrony nor by any combination of these types (Rice, 1997). For this reason, Rice (1997) argued that the step of linearization of ontogenetic trajectories is an important step to carry out in the analysis of ontogenetic trajectories. It can reveal a uniform transformation of ontogenetic trajectories that is meaningfully described by the classical heterochronic categories or non-uniform changes that point out to other processes that should not be disregarded.

The concept of allometry, coined by Huxley & Teissier (1936) refers to the problem of relative growth: what are the 'laws' of growth which underlie the correlations between the changes in the relative dimensions of parts of the body and the changes in overall size? Originally intended to infer dynamic processes of growth, the concept of allometry has been redefined over the years as the statistical correlation of size and shape, or to the effects of size on shape (see discussion by Blackstone 1987; Klingenberg, 1998). The problem of inferring underlying developmental processes from size and shape measurements is a riddle, already addressed by Waddington (1950, p. 511) who remarked "*that the allometry equation has the status, not of a physiological principle, but of a rough and ready shorthand method of description"*. He noted (p.511-513) that *"[o]ne of the most drastic types of simplification is to consider the form as made up simply of two masses and to study the relation between their sizes. This is a procedure which leads to the description of animal form in terms of allometry or 'relative growth'. A rather less drastic simplification along similar lines gives us a description in terms of growth gradients…But it would also suffer from the same limitation, that of being an empirical description with no simple relation to the effective causal system whose nature and modifications constitute the biological problem which we have to understand"*.

One way to partly circumvent these limitations is to use a null theoretical model to examine '*in silico*' the influence of growth processes on morphological changes, as estimated by diverse metrics. Such a model provides a background against which we can compare empirical results. One interest of this theoretical approach is that it can integrate the time/age variable which is unavailable in most empirical studies (see Godfrey & Sutherland, 1995a, b for a discussion). Despite a wealth of studies revealing the influence of diverse factors on growth (e.g. genes, hormones, food, temperature, etc.), much less attention has been devoted to the spatio-temporal mechanisms of growth. Indeed, how the absolute and relative sizes of traits are regulated remains a conundrum (e.g. Nijhout & Emlen, 1998). However, some studies provided a framework to empirically test some simple theoretical hypotheses about how growth dynamics impinge on heterochrony and/or allometry (Godfrey & Sutherland, 1995b, Van der Meulen & Carter, 1995; Nijhout & Wheeler,

1996; Rice, 1997; Nijhout & Emlen, 1998; Stern & Emlen, 1999; Zollikofer & Ponce de León, 2004).

In this paper, we will follow a similar approach to examine the relationships between growth rate, shape and allometries of molluscan shells. After reviewing the previous theoretical approaches devoted to the understanding of molluscan shell geometry and its genesis, we present a free-form vector model simulating allometric shell growth. We investigate how hypothetical growth processes impinge on the derived allometries.

This null model generates various correlations between morphometric variables and provides insights into potential growth-dependent shape changes. Depending on the growth curves, the resulting ontogenetic allometry can be non-linear on a log-log scale. When growth curves depart from exponential functions, the allometric coefficient (slope of the regression line between two traits linear measurements) is time-dependent. This model recalls a much forgotten fact: the allometric coefficient always depends on the instantaneous growth rate and its evolution over time.

This model also illustrates some fundamental properties of accretionary growth, since simple construction rules are sufficient to generate shapes conforming to nearly logarithmic spiral coiling. Ultimately, this model could be used to develop a mathematically data-driven approach where empirical growth curves could be used as inputs in the model. This could assist in testing the hypotheses of growth assumed here. The rejection of these simple hypotheses could help in gaining information on more specific hypotheses of growth in return.

II. Molluscan shell models: from shape to growth

Modelling mollusc shell coiling has a long history rooted in the recognition of the shared logarithmic spiral geometry among mollusc shells (Moseley, 1838; Whitworth, 1862) and other accretionarily growing structures as diverse as horns, claws and teeth (D'Arcy Thompson, 1952; see Skalak, Farrow & Hoger, 1997 for a more recent account on this topic). The simple logarithmic spiral model states that the radius of a logarithmic spiral is an exponential function of the angle of revolution (see Appendix A, Fig. A1). A shell can be graphically constructed by revolving a generating curve (e.g. a circle) about the axis passing through the spiral pole (coiling axis) along a logarithmic trajectory (the generating spiral) (Fig. 1a). The generating curve represents the aperture or more generally the outline of the shell tube sectioned in a plane containing the coiling axis. The generating spiral is the trajectory followed by the aperture centroid. In a logarithmically coiled shell, the diameter of the generating curve increases exponentially at each equal interval of rotation, so that each new growth increment is shape invariant. Thus, each new growth increment is simply an enlarged version of a previous increment. The angle between the tangent of a spiral trajectory at one point of the generating curve and the radius line at this point is a constant (equiangular angle, see Appendix A). For this reason, the logarithmic spiral is also known as the equiangular spiral (D'Arcy Thompson, 1952).

Computer parameterization of shell coiling was first accomplished by Raup (1961) whose approach triggered the emergence of the so-called 'theoretical morphology' research field. In particular, this approach has been used

to compare the morphological diversity of molluscan shell coiling to theoretical possible shell morphologies bounded in a morphospace whose ranges are defined by continuously varying some standardized model parameters (Raup, 1961; Raup & Michelson, 1965; Raup, 1966; McGhee, 1980). Generally, these model parameters are scalars derived from geometric considerations of the simple logarithmic spiral model. For example, the expansion rate of a spiral is defined as the ratio between two linear dimensions (such as spiral radii) separated by a full revolution (Whitworth, 1862; Raup, 1961; Raup, 1966). The expansion rate is the amount of increase in spiral radius relative to the incremental angle of revolution and is proportional to the equiangular angle (see Appendix A). The ratio of whorl radii separated by a full revolution is generally known as the whorl expansion rate (W).

Several models based on the common principle of moving a generating curve along a generating spiral to compute shell surface have been proposed. Differential geometry

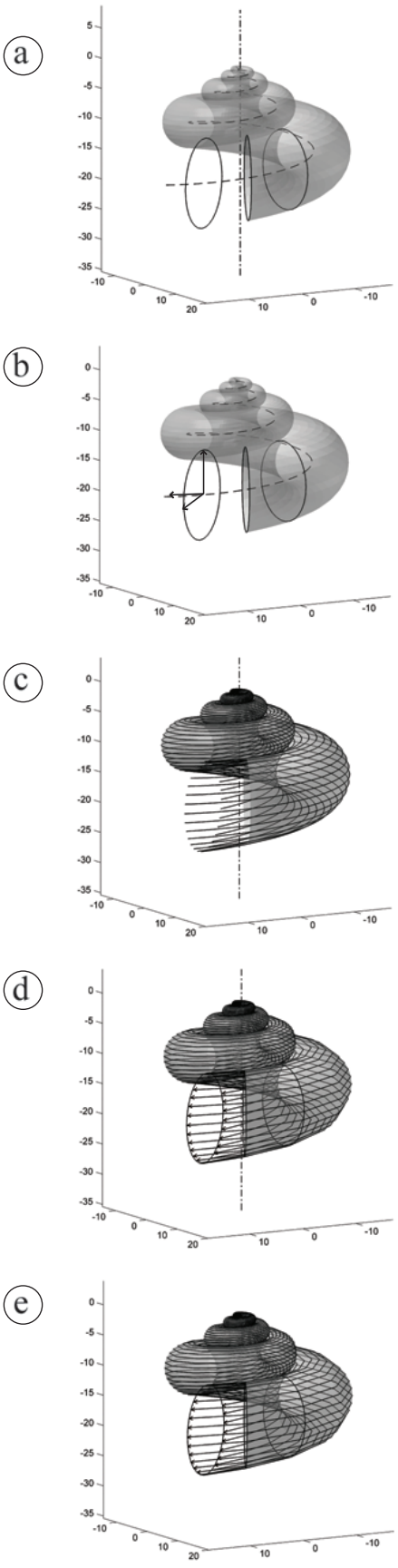


Fig. 1: Different approaches to the simulation of molluscan shell form and growth. Shell surface is let partly translucent to highlight the internal structure. **a**: generating curve models using a fixed reference frame. *Solid lines: generating curves; Dashed line: generating spiral; Dash-dot line: coiling axis.* **b**: generating curve models using a moving reference frame (*arrows*) attached to the generating curve (*solid line*) to simulate the generating spiral (*dashed line*). Contrary to **a**, the generating curve is not restricted to be a logarithmic spiral since such models are based on more general space curve principles. **c**: helicospiral models using a fixed reference frame (*dash-dot line: coiling axis*). Helicospirals (*solid lines*) are simulated independently of one another and each has its own set of parameters values. **d**: multivector helicospiral models using a fixed reference frame (*dash-dot line: coiling axis*). A 'growth matrix' describes the change in the global coordinates of successive apertures. Note that discrete growth is overemphasized by large shell increments. **e**: growth vector model using a moving reference frame. Contrary to **d**, the growth matrix has to be recomputed at each growth step (see Appendix D). These models are based on rigid body motion principles. Note that discrete growth is overemphasized by large shell increments. **a**, **c**, **d** have been described as 'form models' and are generally based on the simple logarithmic spiral model (see Appendix A, Fig. A1), whereas **b** and **e** can be thought of as 'growth-like models' conforming better to the local iterative process of accretionary growth of molluscan shells. Note that fixed axis discrete growth models (**d**) can also be viewed as 'growth-like models' since they attempt to describe the position of growth lines.

approaches flourished from the late 80's as they were becoming less computationally expansive. These models were used to simulate a large variety of shell morphologies. They differed mainly in their use of a fixed reference frame considering a coiling axis (Lovtrup & Von Sydow, 1974; Lovtrup & Lovtrup, 1988; Cortie, 1989; Illert, 1983; Schindel, 1990; Johnston, Tabachnick & Bookstein, 1991; Stone, 1995; Ubukata, 2000; Ubukata, 2003) as opposed to a moving reference frame (Okamoto, 1988; Illert, 1990; Savazzi, 1990) (Fig. 1b). For instance, the latter approach has allowed the simulation of quite unusual shell morphologies (e.g. heteromorph ammonites, Okamoto, 1988; Checa, Okamoto & Keupp, 2002).

Some models did not assume a generating curve, but rather focused on individual helicospirals (McGhee, 1978; Savazzi, 1985; Checa, 1991; Checa & Aguado, 1992). Shell shape was represented by the set of spiral trajectories of given landmarks (e.g. longitudinal ornamentation, Fig. 1c).

Other models represented shell shape as a result of discrete growth steps, using a coiling axis and growth vectors (Fig. 1d, Bayer, 1978; McGhee, 1978; Savazzi, 1985; Illert, 1987). Rather than using the simple spiral model, Ackerly (1989a) devised a discrete growth model based on rigid body motion principles and used a moving reference frame, similar to the Frenet frame proposed at the same time by Okamoto (1988).

The moving reference frame models, the helicospiral multivector models, but also generating curve models allowed the simulation of several kinds of allometries in coiling, aperture size and more rarely aperture shape by introducing predefined perturbations on the parameters defining the shell geometry (Bayer, 1978; McGhee, 1978; McGhee, 1980; Savazzi, 1985; Ackerly, 1989a; Cortie, 1989; Savazzi, 1990; Schindel, 1990; Johnston, Tabachnick & Bookstein, 1991; Checa & Aguado, 1992; Stone, 1996). These models belong to the kinematic class of morphogenetic models, although the time parameter is implicit in the equations. They mimic the movement of structures (generating curve, helicospirals portions or shell increments) in the spatio-temporal space. The successive change in spatial position of these structures generates the shell.

Although relying on similar kinematic approaches, more recent models are more firmly rooted on explicit hypotheses about experimentally testable biological processes. The goal of these molluscan shell models shifted from the morphospace occupation issue to the investigation of the 'biological parameters' (e.g. growth rates) that could account for variation in shell shapes. These models are typically high-dimensional and do not allow the straightforward construction of theoretical morphospaces any more.

For instance, building on previous studies (Lovtrup & Lovtrup, 1988; Hutchinson, 1990), Rice (1998) framed his hypotheses with reference to relative and absolute shell growth rates. In this model, the shell is simulated in a similar manner than fixed-frame generating curve models. However, several 'biologically' relevant parameters are derived from the model, namely the pattern of relative growth rates called aperture map (scalar field of relative growth rates of the aperture's homologous landmarks), an absolute growth rate (scaling of the aperture map), aperture growth rate ('body growth') and various spatial parameters defining the initial conditions and the helicospiral geometry (Fig. 1c).

Although the relationships between shell form and growth rates have been analyzed

earlier (Lovtrup & Lovtrup, 1988; Hutchinson, 1990), Rice's paper marks the first consideration of time as an explicit parameter in shell shape simulation and allows the separation of the magnitude of growth ('rate of calcification') from the direction of growth (aperture map, rate of aperture growth and other parameters related to the definition of helicospirals). Rice (1998) investigated the effects of several hypothetical 'growth laws' on shell shape. His derived 'biological parameters' allowed him to suggest what kind of developmental processes could underlie some shell ontogenies.

Using a growth vector model, Hammer and Bucher (2005) went a step forward in devising a generalized model for molluscan morphogenesis, which encompasses many properties of previously proposed models (Fig. 1e). While Rice (1998) separated shell growth into three kinds of parameters, two scalars defining growth rates (absolute growth rate of shell and aperture), a scalar field representing relative growth rates around the aperture (aperture map) and spatial positions (other scalar parameters), Hammer and Bucher (2005) directly used vectors. Using a local reference frame, Hammer and Bucher (2005) were able to generate an allometry of the aperture that emerged from an implicit growth process. Contrary to the previously discussed models, this allometry is not explicitly defined in the model parameters, but occurs as a consequence of the assumed 'growth rules' and constraints (e.g. constancy of growth vector directions relative to the last computed aperture position, see below). Hammer and Bucher's model (2005) can be viewed as a deeper break through into growth processes, for correlations between various growth directions emerge from the assumed growth process itself and are not stated *a priori*.

Models using fixed reference frames (Figs. 1a, c-d) have been criticized: first, the coiling axis has no biological meaning since in real shells 'something looking like a coiling axis emerges' *a posteriori* as a result of accretionary growth (Ackerly, 1989a, 1989b; Schindel, 1990; McGhee, 1999); second, this approximated axis is not easily located on real shells and small deviations from the 'real axis' (provided that one exists at all) may have large consequences for theoretical and empirical data interpretation; third, many shells do not have a coiling axis at all, or may not have a single coiling axis. Thus, assuming a coiling axis in theoretical or empirical studies may force the interpretations in predefined directions (and perhaps lead to contradictory conclusions, e.g. see McGhee, 1980; Aldridge, 1998; McGhee, 2001). It could also bias the observed morphospace occupation if one is unable to take into account the spiral limit cases (e.g. conical, heteromorph, irregularly coiled) or if parameters are not algebraically independent (as in Raup's model; see Schindel, 1990; McGhee, 1999). In this respect, moving reference models escaped these limitations. But some authors noted that if loosely coiled shells, conical shells or heteromorphs ammonites may be easily analyzed thanks to moving reference models, it was not necessary the case for more tightly or regularly coiled shells which may be straightforwardly represented by fixed reference models (Ackerly, 1989a). Moreover, as the 'phylogeny of shell models' by Stone (1996) made clear, the models using a fixed axis ('form models') facilitate the analysis of morphospace occupation, while moving reference models ('growth-like models') conform better to the accretionary growth but do not usually provide convenient shape description. Assuming a coiling axis is incontestably a convenient premise to

infer the model parameters from real shells or *vice-versa*, provided that one keeps in mind that the subsequent interpretations are somehow tied to such assumptions. Moreover, several studies proposed methods to overcome some problems related to the estimation of the direction and location of a coiling axis and/or spiral pole on real shells (e.g. Ackerly 1989b; Aldridge, 1998; Rice, 1998; Ubukata, 2001), while some others investigated the inverse problem of deriving model parameters from real shells without assuming a coiling axis (e.g. Okamoto, 1988; Ackerly, 1989a). However, if one is rather interested in analysing the ontogenetic variation in shell morphology, it is preferable not to assume a coiling axis *a priori*. As convincingly argued by McGhee (2001, p. 722), *"[i]t is crucial to choose a biologically meaningful coordinate system when taking morphometric measurements from actual organisms if those measurements are to reflect biological realities determined by the actual geometry of the organism under analysis"*.

An issue pervading all studies of form, and shell morphology in particular is: how should spatio-temporal change in the morphology be measured to establish and test hypotheses about underlying growth processes? One approach to this problem consists in constructing a model whose parameters are not theoretically correlated. Then, the estimation of the same parameters in real morphologies allows one to test whether these parameters are biologically correlated. This approach was nicely undertaken by Ackerly (1989a) who, for instance, asked if aperture dilation was developmentally independent of aperture translation. This question can be addressed by Ackerly's model (1989a) but neither by models based on the simple logarithmic model nor by Okamoto's model (1988) which implicitly assume that aperture translation and dilation are geometrically coupled. Thus, *"[t]ranslation and dilation may be biologically coupled, but this result is derived empirically and is not a consequence of the analytical model"* (Ackerly, 1989, p. 162). However, to say that translation and dilation are developmentally coupled is not the same as saying that they represent 'true' distinct biological processes whose empirical correlation has to be found in their joint regulation. They are no more than convenient morphological descriptors that allow us to derive empirical patterns of covariation but unfortunately do not allow us to directly infer the processes underlying such empirically derived patterns of covariation.

To attempt at gaining more information on developmental processes, one could address the inverse problem: what kind of patterns of covariation between morphometric variables do hypothetical processes generate? To address this issue, one has to simulate *'in silico'* plausible processes of growth. Generally, very few is known about which processes of growth could be more likely than others. Then, the alternative is to simulate the simplest processes one can imagine (null hypothesis model). The model parameters do not necessarily represent morphometric variables that could be measured directly or easily on real morphologies. They rather represent simple properties of developmental processes such as timing, interactions among parts, etc. Morphometric variables can be subsequently derived from the simulated morphologies. Then, one can investigate theoretically how changes in hypothetical processes are recorded in the covariation between morphometric variables.

Although the processes are only hypothetical with respect to real processes, the interest of this approach is that it can draw a causal link between variation in processes and variation in the resulting patterns of covariation. But to

conclude something about the variation in real processes given information on the real patterns of covariation forces one to assume that similar patterns of covariation results from similar variation in processes. This is of course a too strong assumption for one could not assume *a priori* a one-to-one correspondence between pattern and process[1]. Although the relationships between pattern and process can be investigated theoretically, the confirmation that some correspondence between them holds can only be gained experimentally by investigating 'directly' the characteristics of the underlying processes. Nevertheless, this approach provides background hypotheses that can assist in devising experiments and in interpreting empirical data[2].

As recalled above, an important characteristic of developmental processes is timing. In high-dimensional models, growth rates are defined as functions of time rather than as functions of size (see Rice, 1998; Hammer & Bucher, 2005). The other models discussed above (Fig. 1), qualified as 'form' and 'growth-like' models by Stone (1996) skipped the time parameter and expressed every parameter as specific growth rates (function of the size of the structure) or relative growth rates (function of other growth rates)[3]. However, if one is interested in going deeper into the description of the underlying developmental processes, time is a requisite both theoretically and empirically. In the next section, we discuss the growth vector model of Hammer & Bucher (2005) in more details and generalize it further, so that it can encompass the vast majority of growth curves and shell shapes.

III. The growth vector model

The growth vector model assumes simple addition of growth vectors, which represent mantle growth during arbitrary (but constant) time steps. The first growth increment defines the so-called growth vectors which are assumed to be constant in direction (relative to the last computed aperture position) during the simulation of a shell (ontogeny). Shell morphology is generated by iteratively adding a growth increment onto the last computed aperture. Each growth increment is obtained by uniformly scaling the growth vectors according to various growth rate curves that simulate mantle growth over time.

As argued by Hammer & Bucher (2005), this model can be viewed in its simplest form as a generalization of the other molluscan shell models discussed in the previous section. It shares obvious similarities with helicospirals multivector models (Bayer, 1978; McGhee, 1978; Savazzi, 1985), with moving reference frame models (e.g. Okamoto, 1988), with the discrete approach of Ackerly (1989a), with the emphasis on growth rates of Hutchinson's study (1990) and Rice's model (1998) and finally with the locally generated aperture allometry of Checa (1991) and Checa & Aguado (1992).

1 Indeed, one has good reasons to think that the contrary holds. For instance, see Zollikofer & Ponce de León (2004, p. 335): *"the multiple and complex effects of process modification on pattern modification even in a simple growth model point to principal limits of inference of process from pattern"*.

2 Most of the time, this approach does not suggest what can be concluded from empirical data but rather suggests what cannot be necessarily concluded from them. More precisely, it clarifies the conditions under which it is justified to extrapolate the results. It can also focus attention on some overlooked assumptions. See below and following chapters.

3 Of course, this situation sounds as a logic choice, for the vast majority of these models were constructed by or for palaeontologists who lack the developmental time anyway.

(1) Definitions

(a) Isometry / allometry

Isometric growth will refer to the simple logarithmic spiral model (Fig. A1, Appendix A and see McGhee, 1999 for a comprehensive and extensive overview). To the contrary, allometric (or anisometric) growth is any departure from the simple logarithmic spiral model. This may be related to the change in aperture shape ('generating curve'), change in aperture rotation, translation, dilation or coiling direction with respect to size or time through ontogeny (longitudinal ontogenetic allometry[4]). Allometric shells are still belonging to the spiral geometry but not to the lognormal spiral anymore.

(b) Shell growth rate

Our theoretical shell growth rate is viewed as an instantaneous growth rate, meaning that it is time-dependent. Shell growth rate at time *t* corresponds to a measure of 'size' of the growth increment that will be added to the shell during a given time step (between *t* and *t+1*). We take the length of the growth vectors as this measure of size. For convenience, it is preferable that our shell growth rate be independent of the first increment size. In all the simulations, shell growth rate at time t_0 (growth increment built between t_0 and t_1) is thus assumed to equal 1. It is as if lengths of all growth vectors had been divided by the length of the first growth vectors between t_0 and t_1. Then, shell growth rate corresponds to the uniform magnification of the initial growth vectors during an unspecified time step (see Appendix B). This time step is kept constant in all simulations and is assumed to be 1. As an increment is added at each time step, two shells generated during a given time interval are necessarily made of the same number of growth increments.

(c) Aperture map / Growth vector map

A consequence of the null hypotheses we assume (see below) is that growth rate is uniform on the aperture outline: it is the same scalar for any point P on the aperture. In other words, the pattern of relative growth rates around the aperture (aperture map *sensu* Rice) always remains constant during the simulation of a shell (ontogeny). The aperture map can be derived from our model as it corresponds to the norm of the growth vectors (at each point P on an aperture) divided by the norm of the growth vector at one reference point (e.g. the closest point to the coiling axis). It seems worth recalling here that in Hammer & Bucher (2005), the 'apertural map' is not equivalent to the previously discussed 'aperture map' of Rice (1998), as it involves a vector field rather than a scalar field. Hence, to avoid confusion, Hammer and Bucher's 'apertural map' will be subsequently denoted as 'growth vector map' when it refers to vectors (growth increment), and 'aperture map' when it refers to the pattern of

4 If measurements represent the time course of ontogeny, allometry is referred to as *growth allometry* (Godfrey & Sutherland 1995a,b) or *ontogenetic allometry* (Cock, 1966; Gould, 1966; Cheverud, 1982b). If these measurements represent a single 'ontogenetic stage' in a single species (e.g. typically adults), allometry is referred to as *static allometry* (Cock, 1966; Cheverud, 1982b; Klingenberg, 1998) or *intra-specific allometry* (Gould, 1966). If these measurements are taken at one 'ontogenetic stage' among phylogenetic lineages, allometry is referred to as *evolutionary allometry* (Cock, 1966; Cheverud, 1982b; Klingenberg, 1998). Gould (1966) referred to evolutionary allometry when the data are supposed to represent a series of ancestors and descendants and *inter-specific allometry* when the data represent contemporaneous species of a clade (sister groups). In Gould's terminology, ontogenetic and evolutionary allometries are dynamic (temporal) while intra- and inter-specific allometries are static (snapshots). *Size allometry* (*sensu* Teissier) qualifies all types of allometry different from growth allometry. Note that ontogenetic allometry can be *longitudinal* or *cross-sectional*. Longitudinal data corresponds to the multiple measurements of the same individual at different ages while cross-sectional data refers to the measurements of several individuals of different size/age (each individual is measured at a single 'ontogenetic stage' or age). In that case, allometry is an average trajectory obtained as a composite from many individuals. Comparisons between successive stages of static allometry (Cock, 1966) can also be viewed as cross-sectional ontogenetic allometry. For a discussion of the relationship between longitudinal ontogenetic allometry, cross-sectional ontogenetic allometry and static allometry at different ontogenetic stages (Cock, 1966), see chap. 4.

relative growth rates around the aperture (norm of growth vectors).

(d) Other measures of growth rates

For convenience, we neglect shell thickness and whorl overlap, but these issues could be investigated using this model with the adequate modifications. Because we assume that shell thickness is constant and uniform, our shell growth rate is equivalent to mantle growth. In a first approximation, the temporal evolution of the incremental surface area produced per given time unit is assumed to be similar to that of shell growth rate (for more details and exceptions, see below). Also, the temporal evolution of shell growth rate would be roughly equivalent to that of shell weight increase per time unit.

(e) Other measures of size

In a first approximation, we assume that the temporal evolution of shell surface area is similar to the 'curvilinear' shell length at one point P on the aperture. The 'curvilinear' shell length at point P is the total length of the shell along the spiral trajectory of this point. The 'curvilinear' shell length at P on the aperture corresponds to the integration of our theoretical growth rate curves. This means that if 'curvilinear' shell length at P follows a logistic curve, shell surface area will also follow a logistic curve. This has been checked to be true in most cases. For instance, should one of the most frequently used proxies of shell size in empirical studies (shell length, shell width) be a logistic function of time, then should shell growth rate (which is the derivative of 'curvilinear' shell length) be a logistic derivative function of time (bell-curved shape). Of course, depending on the allometry, it is expected that the curves describing the temporal evolution of 'curvilinear' shell length, shell area or other measures of size are not linearly proportional to each other.

(f) Experimental versus theoretical growth rate

It must be stressed that our shell growth rate is a theoretical one that corresponds to a normalized instantaneous growth rate per unspecified units of time. This theoretical growth rate should not be confused with an experimentally obtained growth rate expressed in millimeters growth per day or per month for example. But for comparison purposes, it is practical to relate our definition of growth rate to measures of growth rate found in empirical studies. In fact, let say that the length of successive growth increments is maximum at some point M located on the aperture. Then, the growth rate at time t is equal to increment length at M at that time divided by the length of a growth increment taken to be the referential. If the geometry of a growth increment (hereafter growth vector map, see below) is known, shell shapes can be generated with empirically derived growth rate curves.

(2) Growth vector map parameterization

The model is based on a growth vector map which represents the vectors linking homologous points of a growth increment delimited by two (successive) growth lines (see for instance Fig. A2 in Appendix A). Apertures are considered to be planar. Theoretically the description of the relative positions of two apertures as well as their scaling requires only 7 parameters: one three-dimensional rotation (3), one three-dimensional translation (3), and a scaling factor (1).

If one wants to infer which growth vector map parameters should be preferably used to simulate 'real' shells or simply to try to 'guess' which shell shape will be generated

by the model given a particular growth vector map, it is convenient (but not required) to make reference to an external global reference frame whose *z*-axis would presumably coincide with the coiling axis. To do so, we must also describe the location and orientation of the first aperture relative to this global reference frame. Therefore we used 3 supplementary parameters (μ, T_{ix}, T_{iy}, see Fig. A2 in Appendix A for more details).

To define aperture shape, we used circles, ellipses or the digitized contours of 'real' apertures (see Appendix C). In the latter case, two supplementary parameters, representing the maximum dimensions of the aperture along the *z* and *x* axis respectively are used to resize the aperture to the wanted size.

Since the global coordinates of the vectors of the growth vector map will change with successive growth increments as the aperture is rotating, we need to estimate the orientation of the moving aperture for re-orienting the growth vector map accordingly. To do so, we define two local reference axes by using four points on the aperture. These points, called *Left*, *Right*, *Top* and *Bottom* from their position when the first aperture is in the *(xOz)* plane with the coiling direction pointing to the viewer (Fig. A2), are chosen so that the axes they define coincide with the aperture dimensions taken parallel to the *x* and *z* axes and passing through its centroid. For instance, the first axis (*Left-Right*; *Dorso-Ventral* in Hammer & Bucher, 2005) would correspond to the aperture width for a gastropod shell coiling around the *z* axis, whereas the second axis (*Top-Bottom*; *Sinistro-Dextral* in Hammer & Bucher, 2005) would correspond to aperture length. The choice of this coordinate system is arbitrary, as long as it can be defined repeatedly on successive apertures by geometrically homologous points that are in constant structural relation (hereafter called reference landmarks). The two coordinate axes do not have to be orthogonal. Indeed, in a general case, they do not remain orthogonal, even if they are defined as orthogonal in the first aperture for convenience. The normalized cross-product of the two unit directing vectors corresponding to these two axes provides the unit directing vector of the third axis. The sense of the latter vector defines the direction of coiling (clockwise or counter clockwise rotation, corresponding respectively to dextral and sinistral coiling).

In conclusion, we first provide an aperture shape which is scaled (Ro_X, Ro_Z), placed in the *(xOz)* plane and centred on the origin. Along the aperture, *n* points are indexed, including the four reference landmarks. In order to prevent unwanted effects, we interpolate the points on the aperture between these four landmarks to sample them at even space along the aperture outline[5]. The first aperture is then oriented and translated relative to the global reference (μ, T_{ix}, T_{iy}). A second aperture is obtained by a uniform scaling (enlarging) of the first one by a magnification factor (*scale*), followed by three rotations, respectively about *Oy*, *Ox* and *Oz* axes (θ_y, θ_x, θ_z), and finally a three-dimensional translation (T_x, T_y, T_z) (see Fig. A1 in Appendix A, or Appendix C). Any change in one of these 12 parameters is viewed as a change in the growth vector map geometry and may modify the pattern of relative growth rates around the aperture (aperture map). It obviously leads to generating different shell shapes.

Of course, if three-dimensional information on growth increments is available (e.g. CT scans) and if the homology between points on two successive apertures can be defined, the step

5 If points on the aperture are not sampled at even space, the aperture becomes non-planar. The most closely spaced points tend to 'advance slowlier' than the most widely spaced points.

of growth map parameterization is not necessary (although local reference axes are).

A posteriori assessment of the direction of the mean coiling axis, which is convenient for standardized graphical representation, is performed by using a modified version of the stereographic projection method (Ackerly, 1989b).

(3) Growth rules

Shell morphology is generated by iteratively reorienting the growth vector map (*rule 1*), scaling it (*rule 2*) and adding the corresponding growth increment onto the last computed aperture position (see Fig. 2). The algorithm uses a discrete Euler method to generate the shell morphology from the growth vector map (spatial and temporal discretization, see Appendix D for information on the algorithm implementation). The code has been developed using Matlab R14 version 7.01, and is available from the authors upon request[6].

Our ontogenetic null-hypotheses are that at each time step:

1- the directions of the growth vectors are kept constant relative to the last computed aperture;

2- the lengths of the growth vectors are proportional to a given growth rate curve.

This model share obvious similarities with the multivectors model of Bayer (1978) and McGhee (1978, 1999), although this one is more direct as it involves only vector additions and does not assume a coiling axis. Incidentally, *rules 1* and *2* have to be recomputed at each time step, using only the local reference axes of the last computed aperture.

To sum up, our assumptions are:

- Shell growth is discrete. One growth increment is added per constant time interval.
- In one growth increment, the trajectory of any point P on the aperture is a straight line.
- The directions of these straight lines respective to the last computed aperture remain constant along ontogeny.
- Shell growth rate is uniform on the whole aperture (it is a scalar depending on time but not on the position of a point P on the aperture).

Using the same growth vector map, we first investigate the effects of growth rate curves on the direction, speed and pattern of ontogenetic allometry. Then, similar investigations are done by modifying some of the growth vector map parameters (e.g. θ_z), given a particular growth curve.

(4) Morphometrics and statistical analyses

To derive shape variables from the model, we sample the same 'individual' at successive time points (longitudinal data), after reorienting the shell in apertural view. We derive individual ontogenetic trajectories of shape variables currently used in experimental and morphometric studies (shell length, shell width, etc., Figs. 4-7). We also use the geometric morphometrics methods applied on the aperture. As shape changes are linear in the cases we treated here (i.e. shape changes are not restricted to a few of the sampled landmarks that is to say that shape changes are global), we only computed the uniform components of aperture shape changes using the method described in Rohlf & Bookstein (2003).

Allometry of the aperture is recorded as a correlation between derived shape variables

6 See the the screeen shot of the user interface in Appendix E.

(uniform component here) and aperture centroid size, as described by Bookstein (1991). The centroid size is obtained by summing the distances between each landmark and the centroid of the configuration of landmarks. The centroid size is equivalent to the area of a configuration of landmark and is uncorrelated with shape variables in the presence of isometry. In the case of classic morphometric variables (i.e. shell length, shell width), allometry is recorded as a correlation between the logarithm of these distance measures, as it is of standard exercise in many studies.

Another output of the model is the curve of total shell surface area *versus* time. Thus, it can be easily checked whether or not the temporal evolution of the total shell surface area calculated afterwards is similar to the 'curvilinear' shell length used as input in the model.

(5) Growth rate and isometry

The model developed by Hammer & Bucher (2005) gives rise to a non-linear ontogenetic allometry. In their model, growth increment length[7] is supposed to be constant over time (that is, growth rate is constant and equals one) and the directions of growth vectors are kept constant relative to the last computed aperture (*rule 1*). These assumptions imply that the rotation angle between two successive apertures decreases (see Figs. 2a-f). Logarithmic spiral growth would imply that growth increment length be scaled to aperture size. In that case, we would have an exponential relationship between aperture magnification (uniform in its three dimensions) and incremental angle of rotation over time. That is, growth increment length, as well as other linear dimensions, would increase exponentially over time while the rotation angle would remain constant (see Figs. 2a-f and Fig. 4).

Figures 2a-f illustrate both cases with the nearly planispiral growth of a simplistic squared aperture. In the isometric case (Figs. 2a-c), per definition, the aperture shape remains a square. If *rule 1* is respected, it implies that the successive growth increments are homothetic to each other. In other words, their shape remains the same (hence the bold lines in Fig. 2b). However, the lengths of the edges of the square at points *i* are scaled geometrically according to:

$$G_{t,i} = G_{0,i} \times scale^{\,t-1} \qquad (1)$$

Equation 1 means that the *t-th* increment is obtained by uniformly enlarging the first increment G_0 by *scale* to the power of *t* minus 1, *scale* being the size ratio of the first two apertures (between t_0 and t_1). Note that the incremental rotation angle is constant and that aperture length and width increase exponentially (Fig. 4), as do the incremental surface area and the growth rate (growth rate = $G_t / G_0 = scale^{\,t-1}$).

In the second case (Figs. 2d-f), the lengths of the growth vectors remain constant (hence the bold arrows). That is, $G_t = G_0$ and growth rate equals one. Beside the exponentially decreasing incremental rotation angle, note that aperture shape becomes rectangular. Aperture length and width increase linearly, as do the incremental surface area and the 'curvilinear' shell length at any point P on the aperture outline[8]. Note that total (integrated) surface area thus increases quadratically (as the square of aperture length). Compared to the isometric case, the aperture size is smaller in both directions but less compressed in the direction parallel to the mean coiling axis.

7 Lengths of the growth vectors.

8 Curvilinear length at P is the sum of the shell increment lengths at this point.

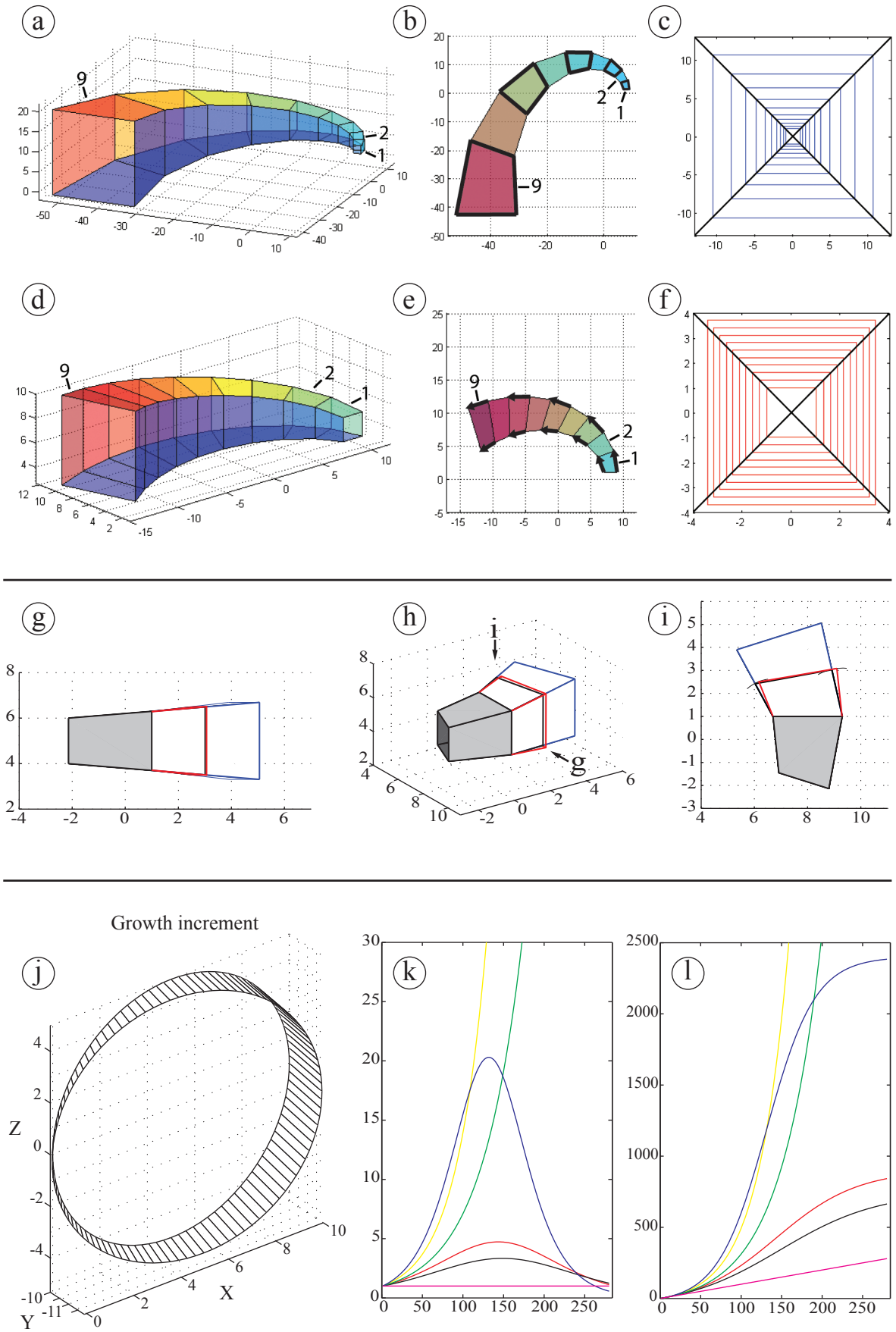
Figures 2g-i illustrate the origin of the observed allometry. Indeed, shall the isometric rule be followed (Equation 1), would each point P on the aperture draw a logarithmic spiral trajectory (thin-black curve). If the successive discrete increments are not scaled according to Equation 1 (for instance in figures 2g-i, the growth rate is $[scale/2]^{t-1}$), then the point P automatically falls outside the spiral trajectory and aperture becomes rectangular. In other words, changing the growth rate will give rise to allometry if *rule 1* is maintained. The only way to make smaller (or larger) increments and nevertheless maintain a squared aperture is to modify the directions of the growth vectors so that each point P remains on the 'spiral' after the corresponding increment. Only that way would the overall shell shape remain the same when the size of growth increments (growth rate) is changed. In figures 2g-i, the blue increment corresponds to the isometric growth of the first increment (as in Figs. 2a-c). The superimposed black increment corresponds to the case where growth rate is twice smaller than isometric growth rate ($[scale/2]^{t-1}$), while *rule 1* is applied (directions of growth vectors remain constant relative to the last aperture position). In this case, the trajectories of the points on the aperture depart from logarithmic spirals. Keeping this growth rate ($[scale/2]^{t-1}$), it is possible to find an increment (red one) for which the points on the aperture do follow a logarithmic spiral and aperture shape remains constant. However, in this case the directions of the growth vectors relative to the previous aperture must be modified.

The allometry just depicted above is dependent on the size of growth increments, and particularly on the rotation angle between two successive apertures (θ_z). Of course, the smaller the θ_z, the smaller the change in the directions of growth vectors for isometry to be maintained when growth rate is different from $scale^{t-1}$. As the allometry depends on θ_z, it is not observed on 'straight growing shells' (perfectly orthocone). In this theoretical limit case, the evolution of growth rate over time would have

Figs. 2a-f: Isometric and anisometric rules of addition of nine growth increments using a squared aperture, a nearly planar growth trajectory and a large angle of rotation around O_z to overemphasize the results of the model assumptions. The local reference axes are defined as one of the vertical sides and one of the horizontal sides of the square. **a, b, c**: isometric case assuming an exponential growth rate where growth vectors lengths are scaled to aperture size at each time step. Successive increments are homothetic (bold line). **d, e, f**: anisometric case assuming a constant growth rate. It means that lengths of growth vectors (arrows) are kept constant throughout ontogeny, as well as their directions relative to the previous aperture. **a, d**: three-dimensional representation of the tube using a square aperture. **b, e**: projection of the tube in the *xOy* plane. Note that in the anisometric case (**d-f**), the rotation angle between two apertures decreases as new increments are added, whereas the rotation angle is invariant in the isometric case (**a-c**). The two obtained spirals are very different, since the geometric progression in the isometric case leads to a relatively more tightly coiled spiral (more rapidly expanding tube) as well as an absolutely larger tube (note the scale) for equal number of increments. **c, f**: projection of ten successive apertures in the same plane. Note that in the anisometric case (**d-f**), the initially squared aperture changes to a rectangle which is taller than wider and the aperture scaling is linear. In the isometric case, aperture scaling is exponential (**a-c**, note the geometric progression) and aperture does not change its shape (the diagonals of the square in **c** are redrawn in **f** for comparison).

Figs. 2g-i: Origin of the observed allometry: the blue increment corresponds to the isometric growth of the first (gray) increment (as in Figs. 2**a-c**). The superimposed black increment corresponds to the case where growth rate is twice smaller than that of isometric growth (*scale*/2), while *rule 1* is applied (directions of growth vectors remain constant relative to the last aperture position). In this case, the trajectories of the points on the aperture depart from logarithmic spirals. Keeping this growth rate (*scale*/2), it is possible to find an increment (red one) for which the points on the aperture do follow a logarithmic spiral and aperture shape remains constant. However, the directions of the growth vectors relative to the previous aperture must be modified. **g**: right view. **h**: 3D view. **i**: top view.

Figs. 2j-l: Inputs of the growth vector model. **j**: growth vector map defining the first shell increment in all the simulations of Figs. 3. Aperture is circular. **k**: growth rate curves used as inputs for the scaling in time of the growth vector map of some shells simulated in Figs. 3. **l**: time evolution of the 'curvilinear' length of the shell at some point of the aperture depending on the growth rate curve assumed in **k**.
green: isometry; **magenta**: constant growth rate; **yellow**: exponential (+) growth rate; **blue**: logistic derivative (+) growth rate; **red**: logistic derivative (iso) growth rate; **black**: logistic derivative (-) growth rate.


a
b
c
d
e
f
g
h
i
j
k
l
Growth increment
X
Y
Z

no influence on the overall shape of the shell, except for a magnification factor (and except for the spacing between successive growth lines). However, when θ_z is different from zero, it can be shown that the resulting allometry increases quadratically with the rotation angle, all else being equal. In the constant growth rate case (Figs. 2d-f), as the rotation angle exponentially decreases and tends towards zero, so does the 'incremental' or 'instantaneous' allometry and the overall allometry tends toward an asymptote. That is, the temporal evolution of apertural shape follows an asymptotic curve (Fig. 5).

(6) Ontogenetic allometry with different growth curves given a growth vector map

Since empirical evidence suggests that instantaneous growth rates often decrease with increasing size in several different groups of molluscs (e.g. Bretos, 1980; Picken, 1980; Guzman & Rios, 1987; Black, Turner & Johnson,1994; Iijima, 2001; Schöne *et al.*, 2002; Schöne *et al.*, 2007), we generalize the model proposed by Hammer & Bucher (2005) to asymptotic growth curves such as logistic curves.

Using the same initial growth vector map (Fig. 2j), different growth rate curves *('exponential isometric'*, *'constant'*, *'exponential +'* and *'bell-shaped'*, see Fig. 2k) are used to simulate shell growth over time (*rule 2*). The corresponding growth curves obtained by integrating the growth rate curves over time are shown in Fig. 2l *('exponential isometric','linear'*, *'exponential'* and *'logistic'* respectively). Figure 3 illustrates the shells generated in each corresponding case, drawn at the same shell length to emphasize shape differences. The morphometric outputs in each respective case are shown in the following Figs. 4-7.

Obviously, the allometry is observed not only in the constant growth rate case (Fig. 5) but in any case where the growth rate curve departs from $scale^{t-1}$ (isometric case, Fig. 4). The further away the growth rate evolves from the isometric growth rate curve, the larger the allometry. Below this curve, the allometry corresponds to a compression in the direction perpendicular to the mean coiling axis (i.e. radially, Fig. 5, Fig. 7). In these cases, the allometric exponent (k) corresponding to the slope of the linear regression of the log-transformed measurements of aperture length[9] against the log-transformed measurement of aperture width[10] is positive. The incremental rotation angle decreases over time and aperture allometry tends toward an asymptote. Above the isometric curve, the allometry corresponds to a radial depression and the allometric coefficient (k) is negative (Fig. 6). The incremental rotation angle increases over time, then it remains constant. As a consequence, the aperture allometry also tends toward an asymptote over time.

Note that the plots of linear measurements (shell length, aperture length) are linear on a log-log scale solely when our growth rate curves are exponential functions of time. In other instances, the plots of linear measurements are not linearized on a log-log scale. The equation proposed by Huxley & Teissier (1936) was specifically proposed for multiplicative growth (exponential growth). In that case, the absolute growth rates of two traits x and y are directly proportional to the size of x and y at each given time t:

$$dx_t / dt = k_1 \times x_t \quad (2)$$

$$dy_t / dt = k_2 \times y_t \quad (3)$$

As a consequence, the ratio of specific growth rates of the two traits x and y is constant over

9 parallel to the 'coiling axis'.

10 perpendicular to the 'coiling axis'.

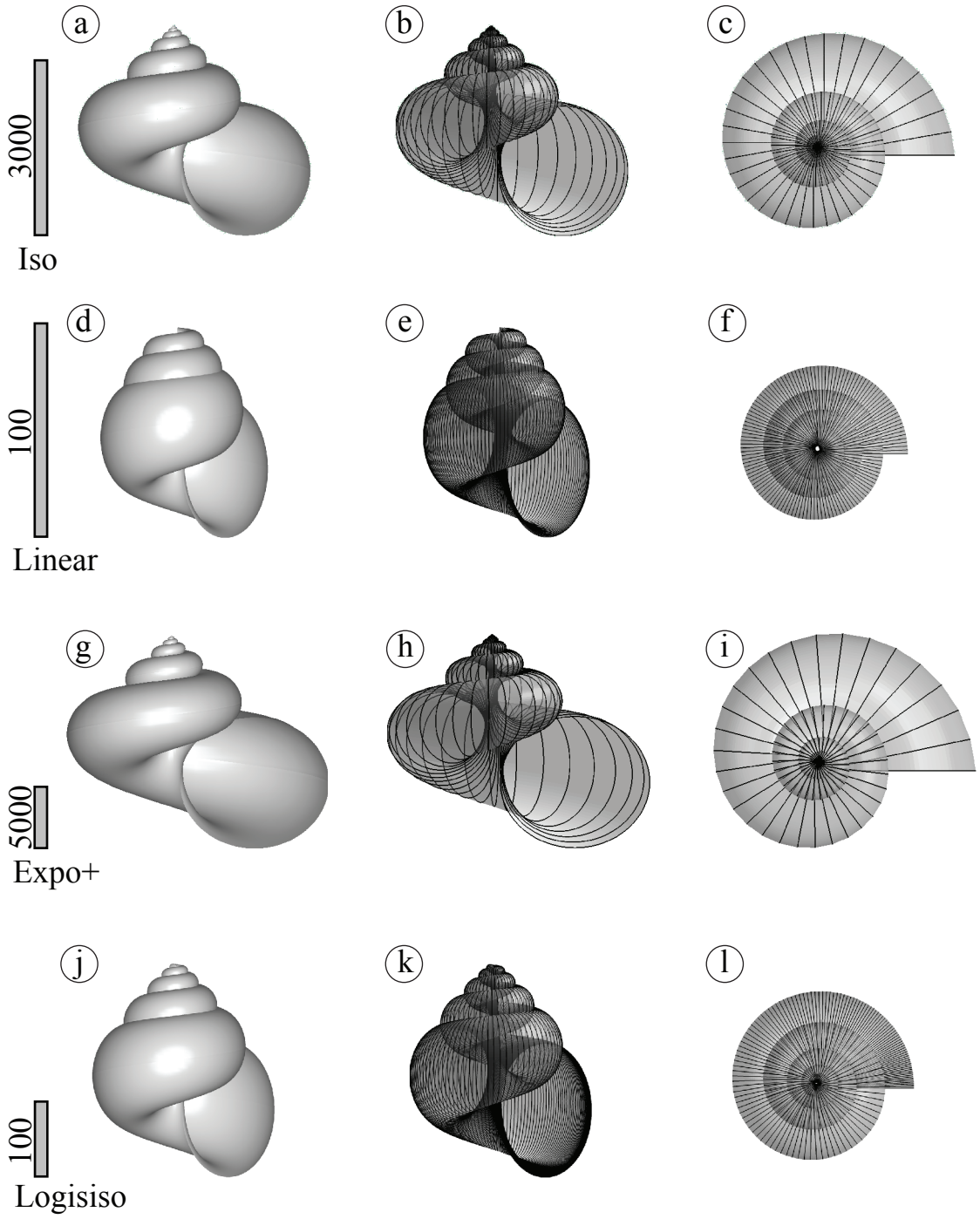


Fig. 3: Shells obtained from different growth rate curves (Fig. 2k), starting from the same growth vector map (Fig. 2j). **a, d, g, j**: apertural view. **b, e, h, k**: translucent apertural view and growth lines. **c, f, i, l**: translucent apical view and growth lines. **a, b, c**: isometric growth. **d, e, f**: constant growth rate. **g, h, i**: exponential (+) growth rate. **j, k, l**: bell-shape growth rate curve starting from isometric conditions.

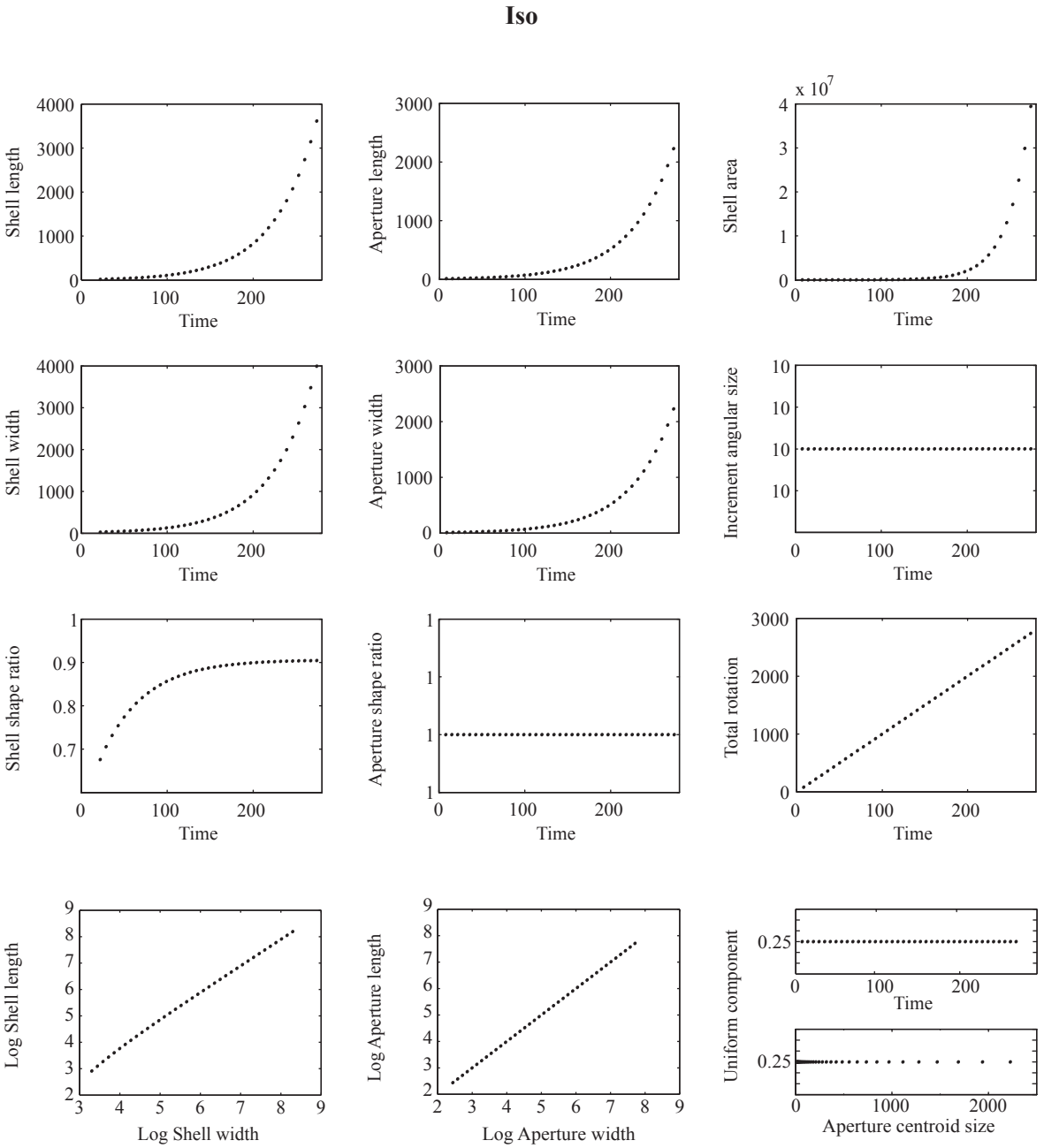


Fig. 4: Morphometric analyses of the shell images of Figs. 3**a**, **b**, **c**. (isometry) using the growth vector map of Fig. 2j.

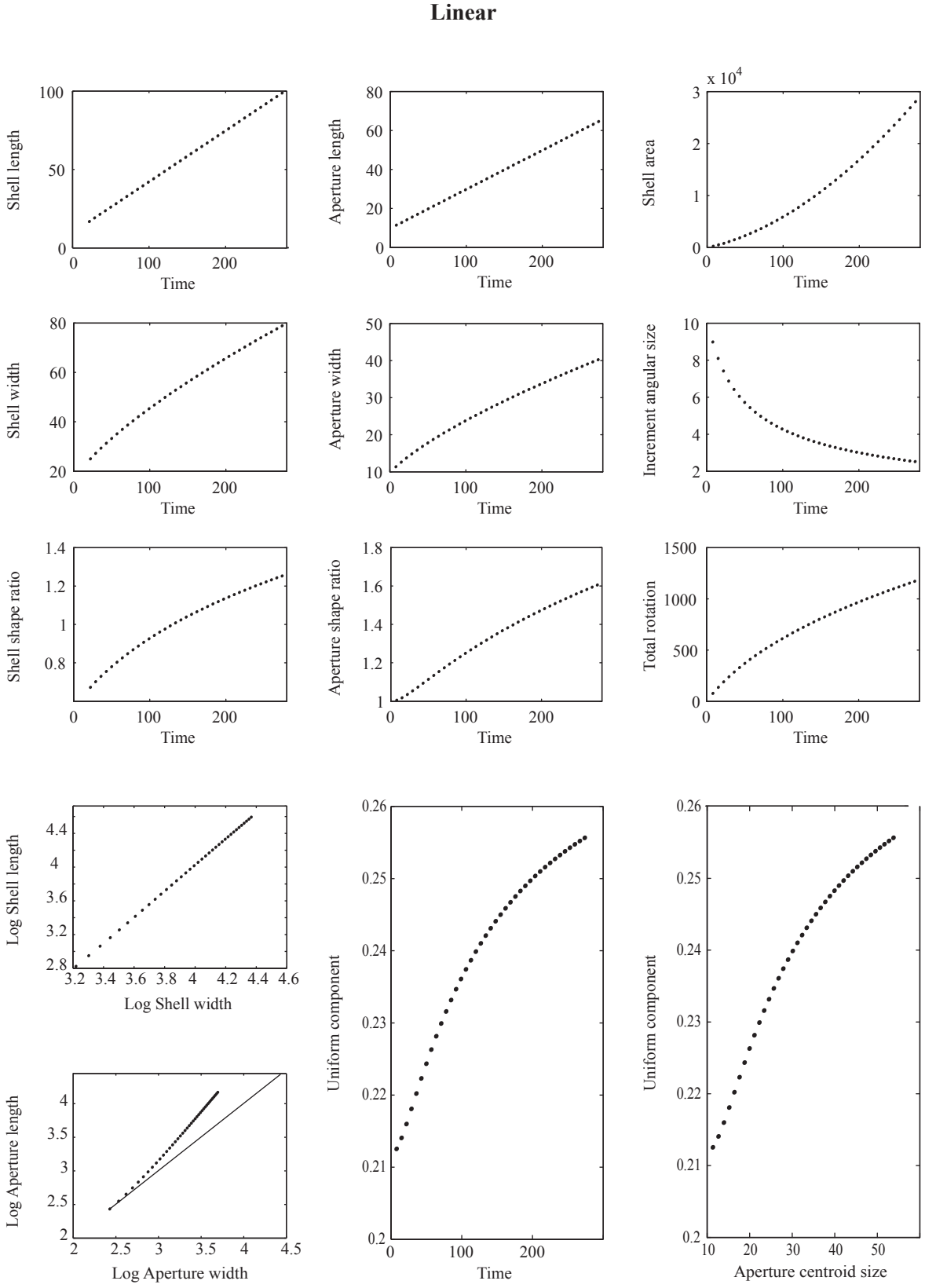


Fig. 5: Morphometric analyses of the shell images of Figs. 3**d**, **e**, **f** (constant growth rate curve) using the growth vector map of Fig. 2j.

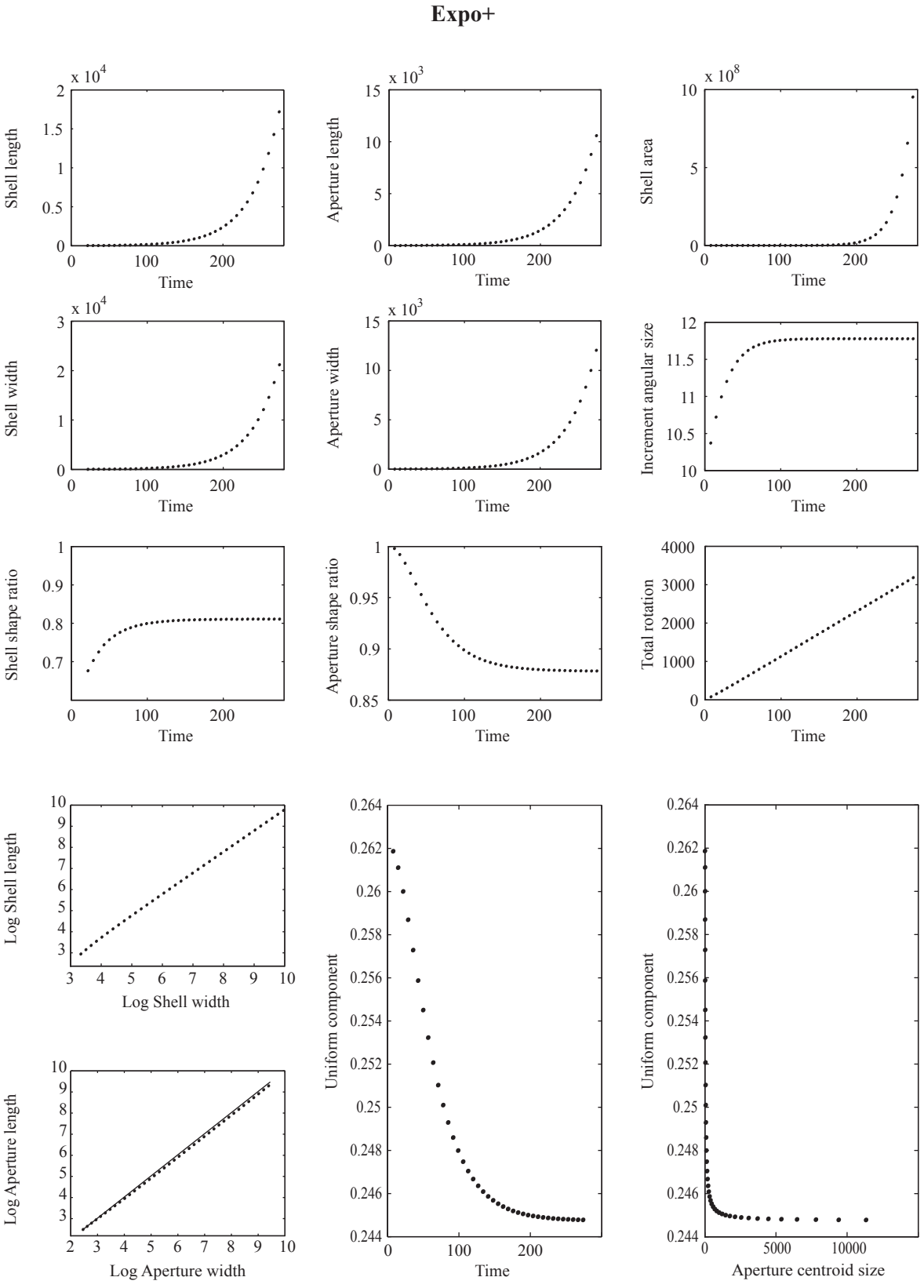


Fig. 6: Morphometric analyses of the shell images of Figs. 3**g**, **h**, **i** (exponential + growth rate curve) using the growth vector map of Fig. 2j.

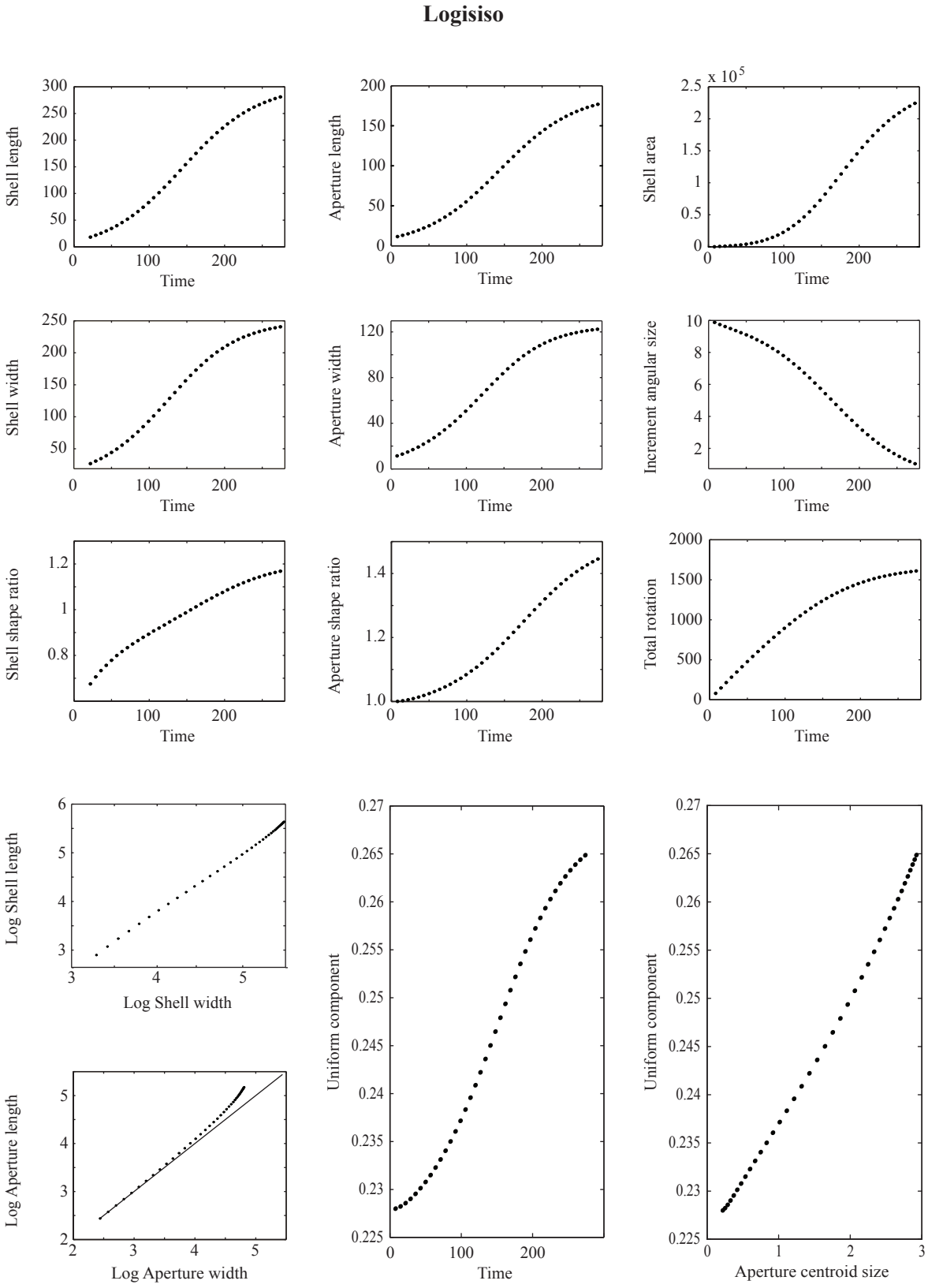


Fig. 7: Morphometric analyses of the shell images of Figs. 3**j**, **k**, **l** (bell shaped growth rate curve starting from isometric conditions) using the growth vector map of Fig. 2j.

time:

$$1/ y_t \times dy_t / dt = k \times 1 / x_t \times dx_t / dt \quad (4)$$

Upon integration over time, Equation 4 yields the classical allometric equation:

$$y_t = b \times x_t^k \quad (5)$$

where b is a scaling coefficient and k is the allometric coefficient.

Equation 5 is linearized on a log-log scale:

$$\log y_t = \log b + k \times \log x_t \quad (6)$$

$$\text{with } k = (dy_t / y_t) / (dx_t / x_t) \quad (7)$$

As long as the ratio of specific growth rates of two traits x and y remains constant over time (Equation 4), the resulting allometric plot will be linear on a log-log scale (Equations 5 & 6) and k is time-independent (Equation 7). This assumption generally holds for growth rate curves which are only simple exponential functions (e.g. 'exponential case' here). In other cases, the ratio of specific growth rates of x and y generally does not remain constant over time and thus k is time dependent. For instance, in our model, if growth rate follows a bell-shaped curve, the resulting allometry is a logistic function of time (Fig. 7). In consequence, the adequate regression of log-transformed linear measurements would require the fitting of two straight lines with different allometric coefficients k before and after the inflexion point. A consequence of such non-linear allometries on a log-log scale is that empirically a regression on adult measurements in a population[11] would result in a different allometric coefficient k than regressions performed on juveniles of a given age[12] and than regressions on the whole ontogenetic sequence[13].

Note also that the plot of shell length *vs*. shell width on a log-log scale is not strictly linear and of slope 1 in the isometric case (Fig. 4). This is so because shell length is measured from the apex to the bottom of the last aperture, as in most empirical studies. For such measurements to provide an allometric coefficient of 1 (isometry), shell length has to be measured from the top of the 'enveloping cone'[14] rather than from the shell apex. As the distance between the top of the 'enveloping cone' and shell apex decreases relatively to shell length as the number of whorl increases, the allometric coefficient tends toward 1 at the end of ontogeny in the isometric case.

Examples considering ratios of measurements are also provided (Figs. 4-7). Shell shape ratio is the ratio between shell length and shell width whereas aperture shape ratio is the ratio between aperture length and aperture width. Such measurements are often used as metrics for shape. But in particular, note that shell shape ratio is dependent on the number of whorls (or time) even in isometrically growing shells at least in the beginning of ontogeny (Fig. 4). These measurements should then be used with care if one wants to compare juvenile shells or a population of snails varying in number of whorls. Only after a certain number of whorls are built[15] can shell shape ratio be truly considered as a shape parameter.

Except for the constant growth rate case and some special cases with logistic growth curves, we do get similar curves for both the 'curvilinear' shell length used as input (Fig. 2l) and the shell surface area computed once shell

11 Static allometry.

12 Static allometry.

13 Ontogenetic allometry.

14 The top of the 'enveloping cone' is the crossing point of the two lines which are tangent to the sides of the shell in apertural view at a point P on the aperture.

15 The number of whorls at which shell shape ratio remains constant in the presence of isometry depends on the shape of the shell. This number will be larger for high spired shells than for low spired shells.

morphology is generated (Figs. 4, 6-7).

The four cases discussed above are indeed a really small subset of a virtually infinite number of possibilities. For instance, it must be understood that the constant growth rate case is only a very special case of exponential growth rate where the magnification factor from one increment to the next (*scale*) is equal to 1. Also by varying the exponential rate growth curves (respectively decreasing or increasing the magnification factor), it is possible to continuously relate the particular isometric case to other cases where aperture allometry is positive or negative respectively. The bell-shaped growth rate curve is also understood as a particular case of exponential growth rate. It is possible for the logistic growth curve to be the same as the isometric exponential growth curve, at least for a certain number of steps in the beginning of a shell simulation (Fig. 7). But the logistic curve can also start below or above the isometric exponential growth curve, thus giving rise to more complex patterns of allometry. Also different sub-cases can be imagined, for instance exponential curves saturating at different rates, Gompertz or von Bertalanffy growth curves. Indeed, any growth rate curve can be used with this model which is fully numeric. For example, seasonal or tidal variations in growth rate could possibly be simulated. This paves the way toward the development of data-driven mathematical models.

(7) Ontogenetic allometry with different growth vector maps given a growth curve

Assuming a particular growth curve (*Expo* -), we now exemplify how varying the incremental angle of rotation (θ_z between 8° to 14°) in the initial growth vector map affects the speed of aperture allometry (Fig. 8). As discussed above, the incremental allometry increases quadratically with the rotation angle around the z axis. The shell starting with a rotation angle of 14° is thus the more allometric over time (allometry is both faster and larger). However, as the number of growth increments are the same in the four simulations of Fig. 8 (400 increments), but incremental surface area increases from left to right, the shell with an initial rotation angle of 14 degrees is the less allometric compared to total shell surface area. Increasing the rotation angle θ_z while keeping other growth vector map parameters constant is equivalent to decreasing Raup's whorl expansion rate (W). Note that the shells have the same length at equal number of growth increments since the absolute translation parameter (T_z) along the 'coiling axis' is kept constant. As a consequence, the overlapping of successive whorls increases from left to right. The other 'rotation' parameters (μ, θ_x, and θ_y) also have an effect on the allometry pattern. For instance, the effect of μ, which corresponds to the initial aperture inclination relative to the 'coiling axis', is partly illustrated in Fig. 8: the temporal evolution of aperture shape ratio shows damped oscillations at the beginning of ontogeny (about the first 200 time steps). Other simulations (not shown) lead us to the conclusion that the larger the initial aperture inclination (μ) the higher the frequency of the damped oscillations and the smaller the resulting allometry.

(8) Triphasic ontogenetic allometry in *Cerion*

The *Cerion* gastropods are well-known for their strongly allometric shell. As described by Gould (1984, p. 218), *"Cerion grows with complex allometries in three phases. It begins with a juvenile shell, triangularly shaped or even button-like in cross section. So different in shape*

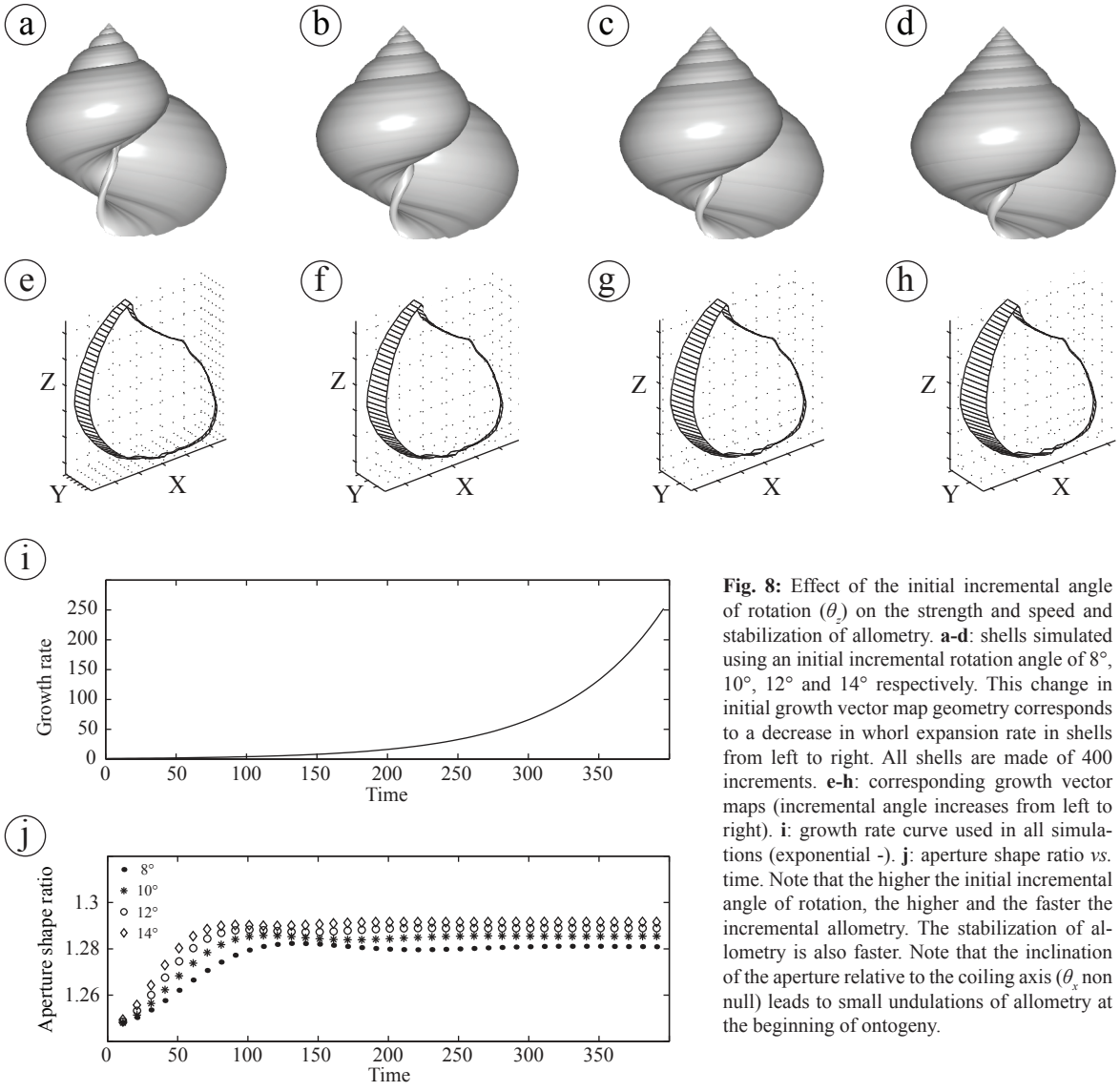


Fig. 8: Effect of the initial incremental angle of rotation (θ_z) on the strength and speed and stabilization of allometry. **a-d**: shells simulated using an initial incremental rotation angle of 8°, 10°, 12° and 14° respectively. This change in initial growth vector map geometry corresponds to a decrease in whorl expansion rate in shells from left to right. All shells are made of 400 increments. **e-h**: corresponding growth vector maps (incremental angle increases from left to right). **i**: growth rate curve used in all simulations (exponential -). **j**: aperture shape ratio *vs.* time. Note that the higher the initial incremental angle of rotation, the higher and the faster the incremental allometry. The stabilization of allometry is also faster. Note that the inclination of the aperture relative to the coiling axis (θ_x non null) leads to small undulations of allometry at the beginning of ontogeny.

are these juveniles from their own adults that unsuspecting malacologists have often placed them in separate genera when working from museum cabinets. In the second or barrel phase of middle ontogeny, Cerion adds height without altering width, converting a juvenile triangle into an adult 'beehive'. In the final phase, growth direction changes again and the final whorl veers toward its own apex, slightly overgrowing the previous whorl before depositing the adult lip".

Rice (1998) generated a *Cerion*-like morphology by assuming that the absolute rate of calcification was proportional to aperture size (rather than aperture growth rate) and was a logistic function of time. From these hypotheses, it follows that shell shape is a function of the growth curve of aperture size over time (logistic in this case). The resulting shell morphology looks like the beehived-shaped shell of *Cerion*: shell growth first follows a (isometric) helicospiral in the exponential phase of the aperture growth curve, then, during the asymptotic phase of aperture growth curve, the shell goes on coiling while maintaining aperture size nearly constant, thus naturally simulating to the 'barrel' phase of *Cerion* growth (Gould, 1984,

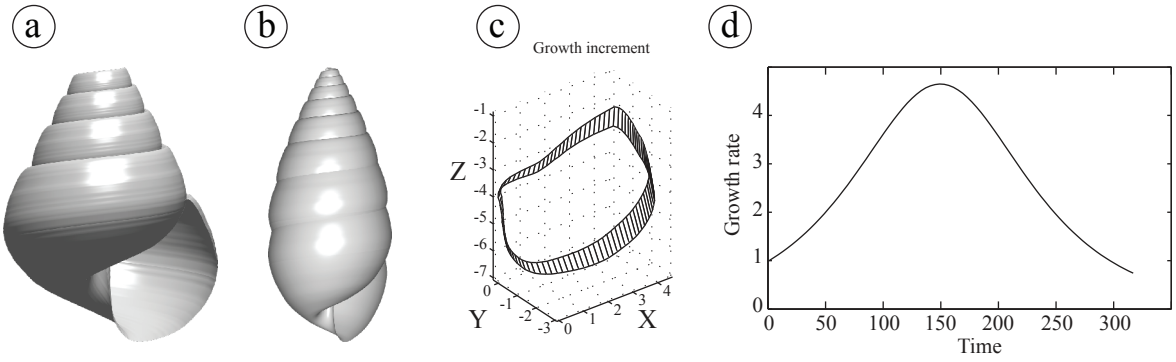


Fig. 9: Cerion shape simulated using a logistic growth curve. **a**: juvenile. **b**: adult. **c**: growth vector map. **d**: growth rate.

1989). The allometry in Rice's example is thus biphasic: the first phase is necessarily isometric whereas the second phase leads to a decrease in shell apical angle (the shell becomes relatively narrower with increasing number of whorls).

In our model, the effect described by Rice (1998) still holds, but major differences are observed. In our case, it is shell growth (total shell surface area or 'curvilinear' shell length), which is assumed to be a logistic function of time (shell growth rate is its derivative) and not shell production rate (growth rate) as in Rice's model (1998). Another difference is that our model implies aperture shape changes, a point that Rice only briefly addresses by noting that aperture shape changes are frequent in gastropods. Indeed, much of the variation in *Cerion* shells seems to result from change of aperture shape during ontogeny. Starting from a triangular aperture digitized from a picture of a juvenile *Cerion* (see Fig. 9a) and assuming a logistic function of shell secretion starting from isometric conditions (see Appendix C), we reproduce a *Cerion*-like morphology where aperture shape becomes more and more elliptic during ontogeny (Figs. 9-10).

Another point of difference between ours and Rice's assumptions is that in our case, growth necessarily stops since growth rate tends toward zero, whereas in Rice's model shell production is theoretically indeterminate but not aperture size which tends toward an asymptote. Moreover, figure 9 shows that one aspect of the third phase of allometry in *Cerion* can be reproduced. It corresponds to the 'constriction' of the aperture at maturity. Indeed, this can happen if the incremental angle of rotation is still relatively large when shell growth rate tends toward zero. Then some few growth increments are added and aperture width may even decrease in size (compare Fig. 7 and Fig. 10). Also, with the exponential growth rate curves (Figs. 6 & 8), it is not necessary for the first 'button' phase of *Cerion* to be isometric as in Rice's study (1998). In fact, the logistic growth curve can start below, above or at the isometric growth curve, thus creating great variability in the possible ontogenetic patterns of allometry. As noted by Gould (1984, p. 217), "*Cerion is a land snail renowned for a diversity of form unparalleled within its group… Previous naturalists responded to this diversity by naming each nuance as a separate species, and producing an unrealistic array of 600 taxa"*. The three phases can be relatively lengthened or shortened in different species. Our model could be used to investigate to which extent simple variation in growth rate could account for the diversity of *Cerion*.

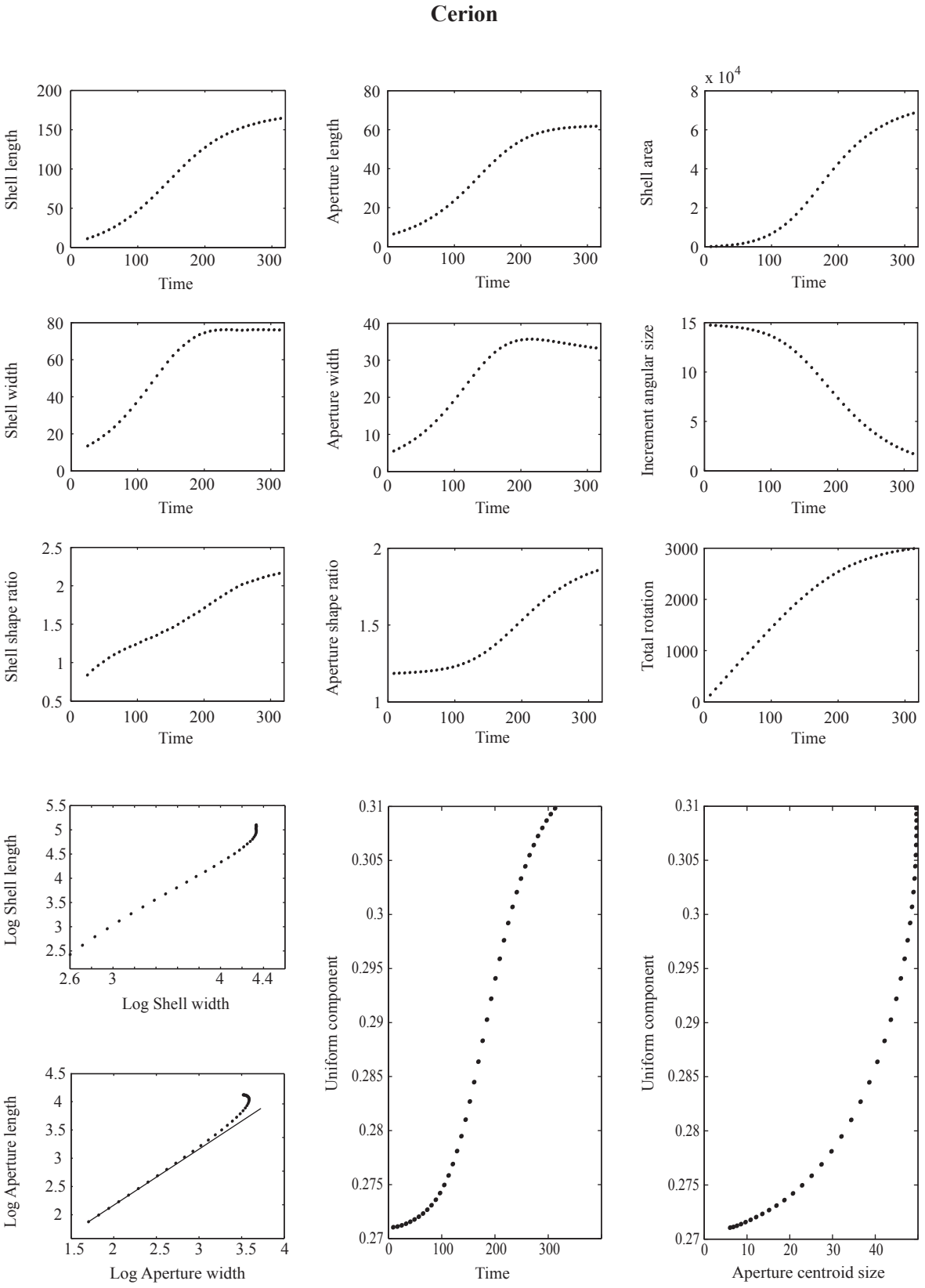


Fig. 10: Morphometric and statistical analyses for the Cerion case (see Fig. 9). Note that the aperture width decreases over time if the rotation angle is still relatively large when growth rate tends toward zero (300 time steps).

IV. Discussion

(1) Further model improvements

(a) Changes at maturity

Changes in coiling parameters at maturity could be explained by simple modifications of this basic model, like more precise choices of growth vector map geometry or by the introduction of new rules and new constraints. For instance, there is no particular reason for *a priori* supposing that growth vector directions should be kept constant relative to the previous aperture (*rule 1*), except that it is the most parsimonious null-hypothesis[16].

Departure from the ideal logarithmic spiral and changes in coiling/aperture shape during the final whorl are common allometries observed at maturity when growth is determinate (mainly in pulmonates but also frequently in prosobranchs, not to mention ammonoids and nautiloids). Such important changes of morphology have intrigued malacologists for many decades. Indeed, the use of allometric equations in the conceptualisation of spiral geometry is almost as old as the logarithmic spiral model based on exponential functions (D'Arcy Thompson, 1952). Also the classic Huxley-Teissier equation (1936) has been applied to ammonoids with shell radius and shell 'curvilinear' length as the two covariate variables (Burnaby, 1966). If the allometric coefficient is less than 1, the equiangular angle (φ, see Appendix A, Fig. A1) decreases and finally becomes zero, leading to uncoiling. The main difference with our approach is that this model is explicitly allometric for the degree of covariation between relative growth rates is stated from the beginning, thus eclipsing the time dimension and discreteness of shell growth process. In our case, supposing that shell growth tends toward an asymptote over time seems sufficient to reproduce some of the well-known allometries (e.g. apertural shape changes, more closely spaced growth lines) often observed at the onset of maturity in molluscs with determinate growth (Fig. 7 and Figs. 9-10). Thus these departures from the equiangular spiral may simply be explained by a decrease in shell growth rate at the onset of maturity, all else being equal. Other possibilities are nevertheless conceivable, for instance those involving changes in the growth directions as shell growth rate decreases. Similarly, for a shell to show no aperture allometry at maturity if rate of shell growth decreases in time (more closely spaced growth lines) necessitates a change in growth vector directions with respect to the previous aperture, thus contradicting our *rule 1* (Figs. 2g-i).

16 A basic counter-argument is that for rib formation, growth vectors directions have to change during ontogeny.

(b) Shell secretion and body growth

Since our model neglects shell thickness, it is assumed that shell growth is equivalent to mantle growth. This assumption is clearly a null-hypothesis in our model, as there is strong empirical evidence that shell and soft body growth can be dissociated in distinct ways among ecomorphs, populations and species (e.g. Appleton & Palmer, 1988; Palmer, 1992; Trussel, 1996). In consequence, assuming that the growth rate of soft tissues simply follows the rate of shell material production (or *vice-versa*) is clearly a simplifying assumption.

Indeed, if mantle and shell growth are decoupled, there is a distinct possibility that an excess in shell material production would result in local thickening with no other consequence for outer shell shape. Such a relative thickening of the shell is conceivable if the mantle stops growing

while the mantle cells continue secreting shell material (Hutchinson, 1990). This phenomenon seems widespread in highly plastic gastropods. In these cases, relative shell thickness has been suggested to be under the influence of ecological factors such as the presence of predators or wave-exposure gradients (e.g. Trussel, 1996). Interestingly, starvation has been shown to be correlated with shell thickness and labral tooth development in *Nucella lapillus* (Cowell & Crothers, 1970) and *Thais lamellosa* (Appleton & Palmer, 1988), probably indicating that shell material goes on being secreted while body growth stops (thus leading to an increase in shell thickness because of the decoupling of shell and soft tissue growth).

But the picture seems even more complicated than this, since the amount of calcium carbonate relative to shell matrix content can also change with overall growth rate[17] (Appleton & Palmer, 1988), not withstanding the remoulding of the interior of the shell wall, a phenomenon widespread in gastropods. If a decoupling between mantle growth and shell growth is allowed in our model, such effects could be simulated.

(c) Influence of growth rhythm on shell shape

In our model, invariance between shell growth rate and shell shape could be reproduced if it is not assumed that a shell increment should be built at each given time step. This choice would lead to disregard the time parameter in the model, as it is the case in most of the shell models discussed so far (except Rice, 1998 and Hammer & Bucher, 2005). Therefore, dividing the time step by some factor between two shell simulations (all else being equal) generates two identical morphologies, except that after a given period of time the sizes (e.g. shell surface areas) differ by an amount proportional to this factor (data not shown).

Another possibility is that the growth vector directions are not kept constant relative to the previous aperture (*rule 1*), but are modified to take into account the evolution of growth rate. In this case overall shell shape could remain 'constant' despite the changes in growth rate but this would require some kind of feedback loop between growth vectors magnification and growth vector directions (see Figs. 2g-i). This would allow the successive addition of many smaller growth increments whose 'cumulative shape' would be indistinguishable from a one-step-built larger growth increment, if one cannot observe the spacing between growth lines. In that case, identical shells (in size and shape but not position of growth lines) can be built, irrespective of the absolute time and steps (growth lines) necessary to grow them. This is particularly true if growth lines are closely spaced, otherwise the shell outline will appear more dissimilar.

The mechanisms underlying shell secretion rhythm are largely a mystery. It has been recognized for long time that shell growth is an intrinsically discrete process. In intertidal gastropods, micro-growth lines in the nacreous layer can reflect daily (or lunar day) increments (e.g. Schöne *et al*., 2007). For instance, in the trochid gastropod *Gibbula cineraria*, Schöne *et al.* (2007) show that shell growth is dependent on the tidal cycles. Bundles of about 14 narrow micro-increments are built between spring tides and micro-growth lines are closely spaced. Bundles of broad micro-increments are built during neap tides and micro-growth lines are barely visible. At the scale of micro-increments, it appears that shell growth rate is higher during neap tides than during spring tides. The bundles

17 defined as the increase in shell weight and body weight.

of micro-increments built during spring tides can be identified as growth lines visible on the outer shell after the fourth whorl (in the second year of growth). The growth duration between these growth lines represent fortnight periods. Moreover, the distance between these successive growth lines decreases over ontogeny, indicating an overall decay of shell growth rate. Some of the growth lines visible on the outer shell represent 'winter' growth lines. Few or no shell is secreted during winter in the species *Gibbula cineraria* and growth rate is maximal from mid spring to mid fall. Thus, shell growth can be discrete and variable at several different time scales: lunar days, tidal cycles and seasons. Of course, this variation is accompanied by the overall ontogenetic decay in growth rates.

Moreover, many shells exhibit distinct discontinuities, so that large shell portions between these discontinuities can be viewed as structurally independent increments often involving different spiral parameters and resulting in a somewhat polygonal shell outline (Bucher & Guex, 1990; Bucher *et al.*, 1996; Bucher, 1997; Chirat & Bucher, 2006). These discontinuities are called growth halts or megastriae. Growth halts are temporary apertures (growth can stop for a several months, especially in the last whorls) and they usually exhibit strong ornaments (spines, tubercles, flares). Some parts of the aperture or spines on the preceding whorl can be partly dissolved with subsequent growth. When growth at the aperture stops (no spiral and no radial growth) the shell can be thickened and some teeth may develop (Spight & Lyons, 1974). The growth vector model could account for these two scales of discontinuities in shell growth (growth lines and megastriae) if one allows shell secretion to stop from time to time and if one can simulate the process of growth halts formation and their often associated pronounced ornamental features, conjointly with the staking of many smaller increments (growth lines).

Then, independently of whether *rule 1* is assumed or not (constancy of growth vector directions relative to the last computed aperture position), two cases can be distinguished:

1- a difference in shell growth rates (this study)

2- a difference in growth rhythm (number of growth halts per time unit).

It seems that both cases are non-exclusive and may be highly variable in living muricid snails (personal observation[18]).

(2) The interest of null hypothesis models

Our null hypothesis model illustrates how simple 'generative rules' of growth can be used to understand some aspects of the generation of forms of specific size and shape. A large part of phenotypic variation and covariation between characters may be explained by the fact that morphogenesis is governed by a small set of basic (sometimes generic) processes that themselves may account for the robustness of the developmental outcome. It is especially true for mollusc shells whose morphogenesis is governed by constructions rules inherent to nearly logarithmic coiling. The main interest of the null hypothesis models of shell morphogenesis is to highlight the rules that may account for some trends in the patterns of intraspecific variation and some recurrent evolutionary patterns.

In our model, since growth rate is assumed to be uniform around the aperture, the aperture map is constant. Under these conditions, aperture shape changes are to be expected if growth rate at a given time is different from the

18 see chapter 5.

ratio between the dimensions of the two preceding apertures (*scale*) while growth directions are kept constant relative to the previous aperture. It means that under these null assumptions, there exist a strong relationship between shell increment size (shell growth rate) and shell increment shape (aperture shape and *scale*). It raises the question of how spiral growth is regulated in various directions. This question can hardly be addressed by geometrical models. The regulation of the magnitude and direction of growth is surely deeply related to the mechanics of the mantle over time, and it is expected that the effect of genes and environmental factors on growth are mediated by these physical factors. The growth vector model does not directly address how the growth vector map is generated and scaled but it must obviously be of valuable help to relate shell shape to the mechanical or chemical aspects of shell morphogenesis (Morita, 1991a, 1991b, 1993, 2003; Hammer & Bucher, 1999). Also, growth vectors can be changed through ontogeny, in an *a priori* way, or in response to shell shape, thus allowing the testing of the 'road-holding hypothesis' (Hutchinson, 1989; Morita, 2003; Hammer & Bucher, 2005).

Intricate relationships between aperture map, aperture shape and coiling have been studied in bivalves theoretically and empirically (Ubukata, 2003). Using a continuous geometrical model with a coiling axis, this author highlights that the aperture map is geometrically dependent on shell convexity (inverse of W) and aperture shape (i.e. his Fig. 5 and 6). He concludes (p. 490) that *"an ontogenetic change of aperture shape is regarded as the inevitable consequence of keeping the basic pattern of the aperture map constant throughout growth, particularly in the species with an inequilateral and noncircular shell form*".

One can imagine three (mutually non exclusive) ways to change the shape of a structure constructed by accretion:

A- modifying the shape of the growth increments (equivalent to: a modification of the aperture map, e.g. Fig. 3 in Rice, 1998; a change of aperture growth rate, e.g. Fig. 8 in Rice 1998; or a transformation of aperture shape);

B- modifying the size of the growth increments (equivalent to modifying shell growth rate, e.g. Fig. 4 in Rice, 1998 or a modification of growth curves, e.g. Fig. 8 in Rice, 1998);

C- modifying the relative arrangement of the growth increments (equivalent to changing the position of aperture map in space, e.g. Fig. 10, 11 and 12 in Rice, 1998; Fig. 8 in Ubukata, 2003).

Among these various possibilities, it is unclear what kind of change could be more frequent in the course of molluscan shell ontogeny or evolution. From the assumptions underlying the growth vector model, we conclude that modifying the size of growth increments and/or the shape of the growth curves (case B) lead to simultaneously changing aperture shape, aperture growth rate (case A) as well as the rotation angle between successive apertures. Modifying the direction of growth vectors relative to the previous aperture (changing *rule 1*) would simulate case C with or without modification of aperture map (case A).

In the absence of experimental studies providing some suggestions, the most mathematically parsimonious assumption is probably that patterns of relative growth rates are more resistant to evolutionary change than absolute growth rates, all else being equal, as Rice (1998) theoretically assumed. But Ubukata's study (2003) well exemplifies how difficult it is to statistically compare high dimensional parameters

like aperture maps. In fact, Ubukata (2003) 'only' qualitatively compared the position of the peak of the aperture map (corresponding to the point on the aperture exhibiting the higher growth rate). But Ubukata's empirical examples tend to point out that extensive variation in the height of the aperture map can be achieved without changing the position of this peak. This ontogenetic increase in the relative growth rates in the ventral region compared to the dorsal region seems to be related to an ontogenetic increase in shell inflation (shell convexity, inverse of W). From this, can we really conclude that "*the basic pattern of the aperture map is generally maintained during ontogeny"* (Ubukata, 2003, p. 489)?

Note also that to define the aperture map during a growth stage, Ubukata (2003) standardized the position of the points along a growth line by the aperture perimeter. In our model, this standardization generates a change in the position of the peak of aperture map over ontogeny when growth is allometric. This comes from the fact that apertural shape changes produce unevenly spaced 'homologuous' points relative to aperture perimeter. The consequences of this standardization on Ubukata's results remain unclear.

(3) On size, shape and growth

Klingenberg (1998) discussed two different frameworks for allometry that have different goals and assumptions. These two frameworks mainly differ in the way they define size and shape. Klingenberg (1998) called the first approach the 'Huxley-Jolicoeur school', which is based on a model of growth dynamics. In this framework, allometry is the pattern of covariation among parts. Shape is loosely defined as the relative size of parts. The allometric equation is based on assumptions upon the relationship between the specific growth rates of the parts under study.

Klingenberg (1998) called the second approach the 'Gould-Mosimann' school, which is based on geometric principles. Mosimann (1970) proposed a mathematical framework to distinguish between size and shape. These definitions rely on vectors of measurements[19] used to compare two objects. Size corresponds to a linear combination of these vectors of measurements. Size is a dimensioned variable which corresponds to the geometric mean of the vectors of measurements: it is a scalar. To the contrary, shape is dimensionless and multivariate. For instance, shape variables correspond to the ratio of vectors of measurements. Two objects are said to be of the same shape if the two vectors of shape variables can be related by a constant. In other words, Mosimann's shape variables correspond to the direction of the vectors of measurements while size variables correspond to the uniform magnification of these vectors of measurements. This is the definition of size and shape which is also endorsed by geometric morphometric methods.

In 1966, Gould (p. 577) expanded the definition of allometry to the *"study of size and its consequences"*. In this framework, allometry simply means that there is some shape changes associated with change in size (isometry is the absence of correlation between size and shape). No special status is given to the Huxley-Teissier allometric equation, except that it generally fits the data well. But to reduce allometry to the correlation between size and shape (without assumptions on growth dynamics) somehow prevents attempts at linking allometry to underlying growth processes (see Blackstone,

19 Mosimann's vectors of measurements are vectors of distances between *n* biologically homologous points.

1987). On the other hand, it points out that a direct analysis of relationships between shape and size variables and their variation in a sample can also be informative. Also, it cautions about the systematic application of a model (like the Huxley-Teissier equation) to the data, which could obscure some interesting 'alternative' interpretation of the data (Mosimann, 1970).

In the definition of our model parameters, we endorsed the Gould-Mosimann's definition of size and shape. We defined growth increment shape as a growth vector field (growth vector map) whereas growth increment size was defined as a scalar (instantaneous growth rate). At the end, our model is able to show how change in size (growth rate) could impinge on change in shape (allometry). By building a theoretical null-model, we were then able to link hypothetical changes in growth processes to changes in shape.

Also, our model is tightly linked to the 'Huxley-Jolicoeur' framework for it can reproduce some of the processes underlying the classical allometric equation. However, our model allows us to question the generality of the assumptions underlying this equation. In particular, before the 70's, it was quite clear that the allometric equation was only meaningful as long as the ratio of specific growth rates of two traits *x* and *y* remained constant over time. But, it seems that this assumption has been much forgotten over the years while the link between allometry and growth rates has been weakened by the geometrical approach of size and shape[20]. Moreover, the systematic logarithmic transformation of linear measurements is usually not much questioned. The success of this transformation in providing relatively good agreement with empirical data has reinforced the impression that the allometric equation was the adequate model in most instances. Yet, it is well known that the logarithmic transformation can render gentle curvatures in allometric relationships invisible (e.g. Godfrey & Sutherland, 1995a). More importantly, Godfrey & Sutherland (1995a) points out that the assumptions underlying the classical allometric equation have often been understated in textbooks, like the one of Batschelet (1979). These authors write (p. 47) that *"Batschelet's mathematical derivation of the power law has been taken as proof that the forms of individual growth curves are irrelevant to the question of the relationships between them. He states (Batschelet, 1979, p. 361), ' the allometric law is not primarily concerned with the speed of growth since time is eliminated [in the derivation of the equation]. An individual may grow as a function of time following an exponential, a logistic or any other law. This leaves the allometric relationship unaffected'"*. Our model points out that this statement is a shortcoming that leads to disregard the very factors (growth curves) that could account for the generation of non-linear allometries. Empirical data highlight that lazy-S growth curves are linked with non-linear ontogenetic allometries (trait against time and/or trait1 *versus* trait2, see Godfrey & Sutherland, 1995a). The consequence is that one should not expect that studies comparing allometry in adults (static allometry) and in juveniles (ontogenetic allometry and/or static allometry across 'growth stages') provide comparable allometric coefficients if trait growth does not follow a simple exponential function.

In conclusion, the 'Huxley-Jolicoeur' and the 'Gould-Mosimann' views on allometry can be combined using growth vector models

20 notwithstanding the scarcity of studies having time data.

and provide distinct evidences. Although both approaches historically lose interest in the timing of growth processes, none of them is inadequate to tackle this aspect of morphogenesis. The 'Huxley-Jolicoeur' approach emphasizes the role of growth dynamics on allometry. But the 'Gould-Mosimann' approach leads to a less drastic characterization of shape than the 'Huxley-Jolicoeur' school, for which shape is only a peripheral concept. Also, the distinction between the magnitude and direction of growth vectors is a technical procedure that facilitates the comparison of the effect of growth vector fields on morphology.

Of course, the differentiation between the magnitude and direction of growth vectors is only justified at the morphological level. For instance, one should not assume that the spatial and scalar components of growth reflect a corresponding dichotomy of processes at the cellular level. This recalls the comment that Klingenberg (1998, p. 84) made within another context: *"although the separation of growth as isometric size increase from all shape changes agrees with our intuitive concept of size and shape based on geometric similarity, it does not reflect a corresponding dichotomy of underlying biological processes"*. In this state of affairs, it appears that biomechanics could provide the link between the various factors acting on growth (genes, hormones, food, temperature, etc) to understand how cell growth is spatio-temporally regulated.

V. Conclusion

The growth vector model assumes simple and direct addition of growth vectors. This model is relatively less restricted than other models (i.e. coiling axis, distinction of aperture shape from spiral growth) and can be viewed as a generalization of earlier proposed models of molluscan shell growth. Because the growth vector model focuses the issue on time, it can highlight a plausible effect of instantaneous growth rate on shell shape. For instance, it shows that shell growth rate is closely related to the geometry of growth increments. Our model highlights these fundamental geometrical properties of logarithmic spirals and raises the question of how growth is regulated in space and time. If it has been widely claimed that time is mandatory to the study of heterochrony, the same can be argued with regards to allometry, especially given the expectation that growth curves are not generally simple power functions of time.

VI. References

Ackerly, S. C. (1989a). Kinematics of accretionary shell growth, with examples from brachiopods and molluscs. *Paleobiology* **15,** 147-164.

Ackerly, S. C. (1989b). Shell coiling in gastropods: analysis by stereographic projection. *Palaios* **4,** 374-378.

Adams, D. C., Rohlf, F. J. & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* **71,** 5-16.

Alberch, P. & Blanco, M. J. (1996). Evolutionary patterns in ontogenetic transformation: from laws to regularities. *International Journal of Developmental Biology* **40,** 845-858.

Alberch, P. & Gale, E. A. (1985). A developmental analysis of an evolutionary trend: digital reduction in Amphibians. *Evolution* **39,** 8-23.

Alberch, P., Gould, S. J., Oster, G. F. & Wake, D. B. (1979). Size and shape in ontogeny and phylogeny. *Paleobiology* **5,** 296-317.

Aldridge, A. E. (1998). Brachiopod outline and the importance of the logarithmic spiral. *Paleobiology* **24,** 215-226.

Appleton, R. D. & Palmer, A. R. (1988). Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proceedings of the National Academy of Sciences of the United States of America* **85,**

4387-4391.

Batschelet, E. (1979). *Introduction to Mathematics for Life Scientists*, Third edition. Springer-Verlag, New York.

Bayer, U. (1978). Morphogenetic programs, instabilities and evolution: a theoretical study. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **156,** 226-261.

Black, R., Turner, S. J. & Johnson, M. S. (1994). The early life history of *Bembicium vittatum* Philippi, 1846 (Gastropoda, Littorinidae). *Veliger* **37,** 393-399.

Blackstone, N. W. (1987). Allometry and relative growth: pattern and process in evolutionary studies. *Systematic zoology* **36,** 76-78.

Bookstein, F. L. (1989). Principal warps: thin-plate splines and the decomposition of deformations. *IEEE Transactions on Pattern Analysis and Machine Intelligence* **11,** 567-585.

Bookstein, F. L. (1991). *Morphometric tools for landmark data*. Cambridge University Press, Cambridge.

Bretos, M. (1980). Age-determination in the keyhole Limpet *Fissurella crassa* Lamarck (Archaeogastropoda, Fissurellidae) based on shell growth rings. *Biological Bulletin* **159,** 606-612.

Bucher, H. (1997). Caractères périodiques et modes de croissance des ammonites: comparaison avec les gastéropodes. *Geobios,* Mémoire spécial **20,** 85-99.

Bucher, H. & Guex, J. (1990). Rythmes de croissance chez les ammonites triasiques. *Bulletin des Laboratoires de Géologie, Minéralogie, Géophysique et du Musée géologique de l'Université de Lausanne* **308,** 191-209.

Bucher, H., Landman, N. H., Klofak, S. M. & Guex, J. (1996). Mode and rate of growth in ammonoids. In *Ammonoid paleobiology* (ed. N. H. Landman), pp. 408-461. Plenum Press, New-York.

Burnaby, T. P. (1966). Allometric growth of ammonoid shells: a generalization of logarithmic spiral. *Nature* **209,** 904-906.

Checa, A. (1991). Sectorial expansion and shell morphogenesis in mollusks. *Lethaia* **24,** 97-114.

Checa, A. & Aguado, R. (1992). Sectorial expansion analysis of irregularly coiled shells: application to the recent gastropod *Distorsio*. *Palaeontology* **35,** 913-925.

Checa, A. G., Okamoto, T. & Keupp, H. (2002). Abnormalities as natural experiments: a morphogenetic model for coiling regulation in planispiral ammonites. *Paleobiology* **28,** 127-138.

Cheverud, J. M. (1982a). Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution* **36,** 499-516.

Cheverud, J. M. (1982b). Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology* **59,** 139-149.

Chirat, R. & Bucher, H. (2006). Shell microstructure and morphogenesis of the ornamentation in *Cymatoceras* Hyatt, 1883, Cretaceous Nautilida: systematic implications. *Lethaia* **39,** 57-64.

Cock, A. (1966). Genetical aspects of metrical growth and form in animals. *Quarterly Review of Biology* **41,** 131-190.

Cortie, M. B. (1989). Models for mollusk shell shape. *South African Journal of Science* **85,** 454-460.

Cowell, E. B. & Crothers, J. H. (1970). Occurrence of multiple rows of teeth in shell of dog-whelk *Nucella lapillus*. *Journal of the Marine Biological Association of the United Kingdom* **50,** 1101-1111.

Godfrey, L. R. & Sutherland, M. R. (1995a). Flawed inference: why size-based tests of heterochronic processes do not work. *Journal of Theoretical Biology* **172,** 43-61.

Godfrey, L. R. & Sutherland, M. R. (1995b). What's growth got to do with it ? Process and product in the evolution of ontogeny. *Journal of Human Evolution* **29,** 405-431.

Gould, S. J. (1966). Allometry and size in ontogeny and phylogeny. *Biological Reviews of the Cambridge Philosophical Society* **41,** 587-640.

Gould, S. J. (1977). *Ontogeny and Phylogeny*. The Belknap Press of Harvard University Press, Cambridge, MA & London.

Gould, S. J. (1984). Covariance sets and ordered geographic variation in *Cerion* from Aruba, Bonaire and Curacao: a way of studying nonadaptation. *Systematic Zoology* **33,** 217-237.

Gould, S. J. (1989). A developmental constraint in *Cerion*, with comments of the definition and interpretation of constraint in evolution. *Evolution* **43,** 516-539.

Guzman, L. F. & Rios, C. F. (1987). Age and growth of the sub-antarctic limpet *Nacella (Patinigera) magellanica magellanica* (Gmelin, 1791) from the Strait of Magellan, Chile. *Veliger* **30,** 159-166.

Hammer, O. & Bucher, H. (1999). Reaction-diffusion processes: application to the morphogenesis of ammonoid ornamentation. *Geobios* **32,** 841-852.

Hammer, O. & Bucher, H. (2005). Models for the morphogenesis of the molluscan shell. *Lethaia* **38,** 111-122.

Hutchinson, J. M. C. (1989). Control of gastropod shell shape: the role of the preceding whorl. *Journal of Theoretical Biology* **140,** 431-444.

Hutchinson, J. M. C. (1990). Control of gastropod shell

form via apertural growth rates. *Journal of Morphology* **206,** 259-264.

Huxley, J. S. & Teissier, G. (1936). Terminology of relative growth. *Nature* **137,** 780-781.

Iijima, A. (2001). Growth of the intertidal snail, *Monodonta labio* (Gastropoda, Prosobranchia) on the Pacific coast of central Japan. *Bulletin of Marine Science* **68,** 27-36.

Illert, C. (1983). The mathematics of gnomonic seashells. *Mathematical Biosciences* **63,** 21-56.

Illert, C. (1987). Formulation and solution of the classical seashell problem. 1. Seashell geometry. *Nuovo Cimento Della Societa Italiana Di Fisica D - Condensed Matter Atomic Molecular And Chemical Physics Fluids Plasmas Biophysics* **9,** 791-814.

Illert, C. (1990). *Nipponites mirabilis*: a challenge to seashell theory. *Nuovo Cimento Della Societa Italiana Di Fisica D - Condensed Matter Atomic Molecular And Chemical Physics Fluids Plasmas Biophysics* **12,** 1405-1421.

Johnston, M. R., Tabachnick, R. E. & Bookstein, F. L. (1991). Landmark-based morphometrics of spiral accretionary growth. *Paleobiology* **17,** 19-36.

Klingenberg, C. P. (1998). Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biological Reviews* **73,** 79-123.

Klingenberg, C. P. & Froese, R. (1991). A multivariate comparison of allometric growth patterns. *Systematic Zoology* **40,** 410-419.

Lovtrup, S. & Lovtrup, M. (1988). The morphogenesis of molluscan shells: a mathematical account using biological parameters. *Journal of Morphology* **197,** 53-62.

Lovtrup, S. & Sydow, B. V. (1974). D'Arcy Thompson's theorems and the shape of the molluscan shell. *Bulletin of Mathematical Biology* **36,** 567-575.

McGhee, G. R. (1978). Analysis of shell torsion phenomenon in Bivalvia. *Lethaia* **11,** 315-329.

McGhee, G. R. (1980). Shell form in the biconvex articulate Brachiopoda: a geometric analysis. *Paleobiology* **6,** 57-76.

McGhee, G. R. (1999). *Theoretical Morphology*. Columbia University Press, New York.

McGhee, G. R. (2001). The question of spiral axes and brachiopod shell growth: a comparison of morphometric techniques. *Paleobiology* **27,** 716-723.

McKinney, M. L. & McNamara, K. J. (1991). *Heterochrony: the evolution of ontogeny*. Plenum Press, New York.

Morita, R. (1991a). Finite element analysis of a double membrane tube (DMS-tube) and its implication for gastropod shell morphology. *Journal of Morphology* **207,** 81-92.

Morita, R. (1991b). Mechanical constraints on aperture form in gastropods. *Journal of Morphology* **207,** 93-102.

Morita, R. (1993). Development mechanics of retractor muscles and the "Dead Spiral Model" in gastropod shell morphogenesis. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **190,** 191-217.

Morita, R. (2003). Why do univalve shells of gastropods coil so tightly? A head-foot guidance model of shell growth and its implication on developmental constraints. In *Morphogenesis and Pattern Formation in Biological Systems: Experiments and Models* (ed. T. Sekimura, S. Noji, N. Ueno and P. K. Maini), pp. 345-354. Springer-Verlag, Tokyo.

Moseley, H. (1838). On the geometrical forms of turbinated and discoid shells. *Philosophical Transactions of the Royal Society of London* **128,** 351-370.

Mosimann, J. E. (1970). Size allometry: size and shape variables with characterizations of lognormal and generalized gamma distributions. *Journal of the American Statistical Association* **65,** 930-945.

Nijhout, H. F. & Emlen, D. J. (1998). Competition among body parts in the development and evolution of insect morphology. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 3685-3689.

Nijhout, H. F. & Wheeler, D. E. (1996). Growth models of complex allometries in holometabolous insects. *American Naturalist* **148,** 40-56.

Okamoto, T. (1988). Analysis of heteromorph ammonoids by differential geometry. *Palaeontology* **31,** 35-52.

Palmer, A. R. (1992). Calcification in marine mollusks: how costly is it? *Proceedings of the National Academy of Sciences of the United States of America* **89,** 1379-1382.

Picken, G. B. (1980). Distribution, growth, and reproduction of the antarctic limpet *Nacella* (*Patinigera*) *Concinna* (Strebel, 1908). *Journal of Experimental Marine Biology and Ecology* **42,** 71-85.

Raff, R. A. & Wray, G. A. (1989). Heterochrony: developmental mechanisms and evolutionary results. *Journal of Evolutionary Biology* **2,** 409-434.

Raup, D. M. (1961). The geometry of coiling in gastropods. *Proceedings of the National Academy of Sciences of the United States of America* **47,** 602-609.

Raup, D. M. (1966). Geometric analysis of shell coiling: some general problems. *Journal of Paleontology* **40,** 1178-1190.

Raup, D. M. & Michelson, A. (1965). Theoretical morphology of the coiled shell. *Science* **147,** 1294-1295.

Rice, S. H. (1997). The analysis of ontogenetic trajectories: when a change in size or shape is not heterochrony. *Proceeding of the National Academy of Sciences of United States of America* **94,** 907-912.

Rice, S. H. (1998). The bio-geometry of mollusc shells. *Paleobiology* **24,** 133-149.

Rohlf, F. J. & Bookstein, F. L. (2003). Computing the uniform component of shape variation. *Systematic Biology* **52,** 66-69.

Rohlf, F. J. & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39,** 40-59.

Roth, V. L. & Mercer, J. M. (2000). Morphometrics in development and evolution. *American Zoologist* **40,** 801-810.

Savazzi, E. (1985). Shellgen: a basic program for the modeling of molluscan shell ontogeny and morphogenesis. *Computers & Geosciences* **11,** 521-530.

Savazzi, E. (1990). Biological aspects of theoretical shell morphology. *Lethaia* **23,** 195-212.

Schindel, D. E. (1990). Unnoccupied morphospace and the coiled geometry of gastropods: architectural constraint or geometrical covariation? In *Causes of evolution: a paleontological perspectives* (ed. R. M. Ross and W. D. Allmon), pp. 270-304. University of Chicago Press, Chicago.

Schöne, B. R., Lega, J., Flessa, K. W., Goodwin, D. H. & Dettman, D. L. (2002). Reconstructing daily temperatures from growth rates of the intertidal bivalve mollusk *Chione cortezi* (northern Gulf of California, Mexico). *Palaeogeography Palaeoclimatology Palaeoecology* **184,** 131-146.

Schöne, B. R., Rodland, D. L., Wehrmann, A., Heidel, B., Oschmann, W., Zhang, Z. J., Fiebig, J. & Beck, L. (2007). Combined sclerochronologic and oxygen isotope analysis of gastropod shells (*Gibbula cineraria*, North Sea): life-history traits and utility as a high-resolution environmental archive for Kelp forests. *Marine Biology* **150,** 1237-1252.

Skalak, R., Farrow, D. A. & Hoger, A. (1997). Kinematics of surface growth. *Journal of Mathematical Biology* **35,** 869-907.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry: the principles and practice of statistics in biological research*, 3rd edition. Freeman, New York.

Spight, T. M. & Lyons, A. (1974). Development and functions of the shell sculpture of the marine snail *Ceratostoma foliatum*. *Marine Biology* **24,** 77-83.

Stern, D. L. & Emlen, D. J. (1999). The developmental basis for allometry in insects. *Development* **126,** 1091-1101.

Stone, J. R. (1995). CerioShell: a computer program designed to simulate variation in shell form. *Paleobiology* **21,** 509-519.

Stone, J. R. (1996). The evolution of ideas: a phylogeny of shell models. *American Naturalist* **148,** 904-929.

Stone, J. R. (1997). The spirit of D'Arcy Thompson dwells in empirical morphospace. *Mathematical Biosciences* **142,** 13-30.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Trussell, G. C. (1996). Phenotypic plasticity in an intertidal snail: the role of a common crab predator. *Evolution* **50,** 448-454.

Ubukata, T. (2000). Theoretical morphology of hinge and shell form in Bivalvia: geometric constraints derived from space conflict between umbones. *Paleobiology* **26,** 606-624.

Ubukata, T. (2001). Morphological significance of the orientation of shell coiling and the outline of "the aperture" in bivalve molluscs. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **221,** 249-270.

Ubukata, T. (2003). Pattern of growth rate around aperture and shell form in Bivalvia: a theoretical morphological study. *Paleobiology* **29,** 480-491.

Van Der Meulen, M. C. H. & Carter, D. R. (1995). Developmental mechanics determine long bone allometry. *Journal of Theoretical Biology* **172,** 323-327.

Waddington, C. H. (1950). The biological foundations of measurements of growth and form. *Proceedings of the Royal Society of London Series B - Biological Sciences* **137,** 509-515.

Webster, M. & Zelditch, M. L. (2005). Evolutionary modifications of ontogeny: heterochrony and beyond. *Paleobiology* **31,** 354-372.

Whitworth, W. A. (1862). The equi-angular spiral, its chief properties proved geometrically. *Messenger of Mathematics* **1,** 5-13.

Zelditch, M. L. (1988). Ontogenetic variation in patterns of phenotypic integration in the laboratory rat. *Evolution* **42,** 28-41.

Zollikofer, C. P. E. & Ponce de Leòn, M. S. (2004). Kinematics of cranial ontogeny: heterotopy, heterochrony, and geometric morphometric analysis of growth models. *Journal of Experimental Zoology Part B - Molecular and Developmental Evolution* **302B,** 322-340.

VII. Appendices

Appendix A: The simple logarithmic spiral model

The following appendix outlines the basis of the 'simple logarithmic spiral model' and the derivation of Raup's whorl expansion *W*.

A logarithmic spiral is a curve whose radius increases exponentially with the angle of revolution. Then, the polar graph of a logarithmic spiral in two dimensions (r, θ) is described as the exponential function:

$$r = a \times \exp(b \times \theta) \quad \text{A.1}$$

where r is the length of the radius vector linking any point P of the curve to the origin; θ is the angle in radians between the x-axis and the radius vector r; a and b are constants (see Fig. A1).

The rate of change of the spiral radius r relative to the angle of revolution θ is:

$$dr / d\theta = a \times b \times \exp(b \times \theta) = b \times r \quad \text{A.2}$$

$$\text{Thus } b = (dr / d\theta) / r \quad \text{A.3}$$

The angle between a radius of the spiral at a point P (r, θ) and the tangent to the curve at this point is called the equiangular angle (Φ). It is given by:

$$\Phi = \tan^{-1} [r / (dr / d\theta)] = \tan^{-1}(1 / b) = \cot^{-1}(b) \quad \text{A.4}$$

$$\text{Thus } b = \cot(\Phi) \quad \text{A.5}$$

As b tends toward 0, Φ tends toward $\pi/2$, and the spiral tends toward a circle.

The expansion rate of the spiral is defined as the ratio of two linear dimensions over one full revolution (2π). Taking spiral radii as the linear dimension:

$$W = r_{2\pi} / r_0 \quad \text{A.6}$$

For two radius r_1 and r_2 rotated by $\Delta\theta = \theta_2 - \theta_1$:

$$W = (r_2 / r_1)^{2\pi / \Delta\theta} \quad \text{A.7}$$

The whorl expansion rate can be shown to be proportional to b. Taking the natural logarithm of both sides of Equation (I.7), we obtain:

$$\ln W = 2\pi / \Delta\theta * \ln(r_2 / r_1) \quad \text{A.8}$$

which is equivalent to:

$$\ln W = 2\pi * [\ln(r_2) - \ln(r_1)] / \Delta\theta = 2\pi * \Delta(\ln r) / \Delta\theta \quad \text{A.9}$$

Allowing the difference values $\Delta\theta$ to be infinitesimally small:

$$\ln W = 2\pi * d(\ln r) / d\theta \quad \text{A.10}$$

As $d(\ln r) = dr/r$:

$$\ln W = 2\pi * (dr / r / d\theta) = 2\pi * b \quad \text{A.11}$$

$$\text{or } b = \ln W / (2\pi) \quad \text{A.12}$$

In Cartesian coordinates, the equation of the spiral (I.1) becomes:

$$x = r \cos\theta = a \cos\theta \exp(b \times \theta) \quad \text{A.13}$$

$$y = r \sin\theta = a \sin\theta \exp(b \times \theta) \quad \text{A.14}$$

Figure A1 shows that for the two last marked radii:

$r_1 = 30$ and $\theta_1 = 300$ °

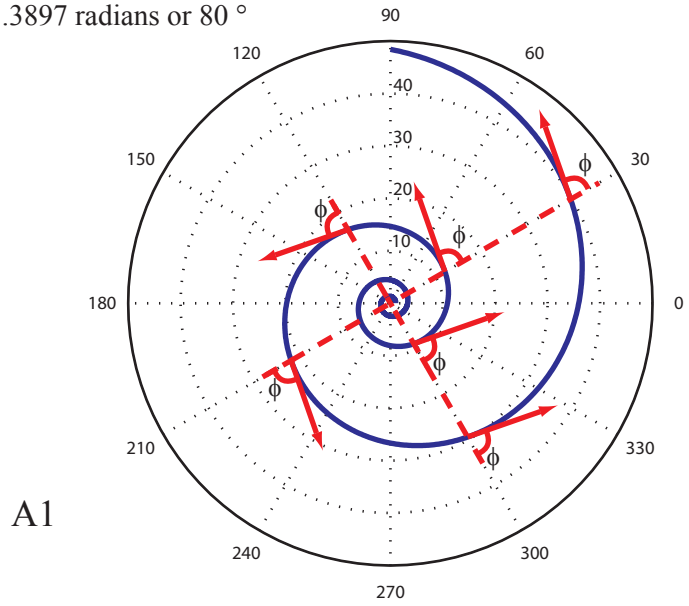
$r_2 = 40$ and $\theta_2 = 390$ °

$\Delta\theta = 390\text{-}300 = 90°$ or $\Delta\theta = \pi/2$ radians

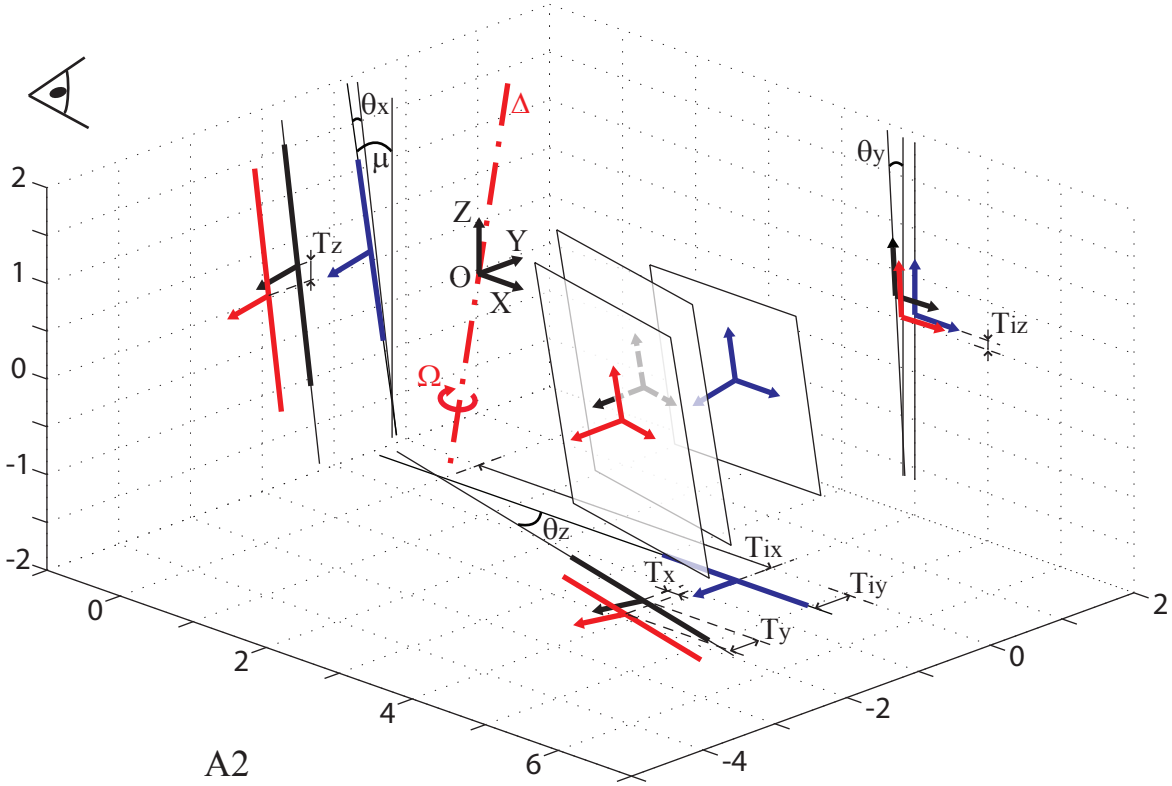
$W = (40/30)^{2\pi/(\pi/2)} = 3.1605$ (A.7)

$b = \ln W / (2\pi) \approx 0.1831$ (A.12)

$\Phi = \cot^{-1}(b) \approx 1.3897$ radians or 80 ° (A.4)



A1



A2

Fig. A1: Planar logarithmic spiral (blue) in polar coordinates (r, θ) illustrating the constancy of the equiangular angle (Φ) defined as the angle between a radius of the spiral at one point of the spiral and the tangent to the curve at this point (red). The radial dot lines indicate the values of θ in degrees. The concentric dot lines indicate the length of the radius vectors.

Fig. A2: 12 parameters are used to define a growth vector map.

Appendix B: Growth curves

This appendix outlines the parameters used in the equations describing the growth rates curves.
In the exponential growth rate case, the instantaneous growth rate is given by:

$$\Delta L_t / \Delta t = L_0 \times \exp(r \times (t\text{-}1)) \quad \text{B.1}$$

$\Delta L_t / \Delta t$ is the length of growth vectors between time t and time t-1 (Δt is set to 1*)*. L_0 (initial size) is set to 1 in all simulations. r is a constant usually known as the 'intrinsic growth rate'.

Upon integration, the growth curve is:

$$L_t = L_0 / r \times \exp(r \times (t\text{-}1)) \quad \text{B.2}$$

where L_t is the shell curvilinear length at time t.

In the isometric case, r = log (*scale*) where *scale* is the size ratio of the two apertures defining the first growth increment.
Then, the length of growth vectors (G_t) at time t are given as:

$$G_t = G_0 \times scale^{(t\text{-}1)}$$

which is equivalent to: $G_t = G_0 \times \Delta L_t / \Delta t$ with $\Delta L_t / \Delta t$ given by B.1.

In the *Expo* – and *Expo* + cases, r is inferior or superior respectively to log (*scale*).

The constant growth rate case ('linear' growth) is a sub-case of exponential growth with r = 0, thus $G_t = G_0$.

Logistic growth curve is simulated using the equation:

$$L_t = K / [1 + (\mathrm{K} / L_0\text{-}1) \times \exp(-r \times (t\text{-}1))] \quad \text{B.3}$$

where K is the asymptotic size, L_0 (initial size) is set to 1, and r is the intrinsic growth rate. For the derivative of this curve to be continuous at t_{infl}, we set:

$$K = 2 \times L_0 \times \exp(r \times (t_{infl}\text{-}1)) \quad \text{B.4}$$

The bell shaped growth rate curve ($\Delta L_t / \Delta t$) is obtained by deriving the previous equation. Thus, it is defined from three parameters (L_0, r, t_{infl}). As previously, $G_t = G_0 \times \Delta L_t / \Delta t$ with $\Delta L_t / \Delta t$ given by B.3.
If r is equal to log (*scale*), the instantaneous growth rate starts at values equal to the isometric case (*Logisiso*). At some time t, the instantaneous growth rate becomes inferior to the isometric growth rate curve. In that case, growth is isometric at the beginning of a simulation and then become similar to the *Expo* – case. If r is inferior or superior to log(*scale*) from the start of a simulation the allometric patterns are more complicated (data not shown).

Appendix C: Model parameters

Parameters \ Fig.	Figs. 3-7	Figs. 9, 10	Fig. 8
Aperture shape	Circle	Juvenile Cerion	Littorina
RoX	10	5	5
RoZ	10	4.5	5
Tix	-10	-10	-10
Tiy	0	0	0
Tiz	0	0	0
μ (°)	0	-10	-10
scale	1.02	1.015	1.016
θx (°)	0	0	0
θy (°)	0	0	0
θz (°)	10	15	8 / 10 / 12 / 14
Tx in % of *RoX*	-1	-2	-1
Ty in % of *RoX*	-1	-1	-1
Tz in % of *RoZ*	-1.5	-3.5	-2
Initial size	1	1	1
Growth rate parameter *r*: $Y(t)=\exp(r\times(t-1))$ except for logistic	Figs. 3a, b, c & Fig. 4: 0.019803 = log(1.02) (´iso´) Figs. 3d, e, f & Fig. 5: 0 = log(1) < log(1.02) (´linear´) Figs. 3g, h, i & Fig. 6: 0.027 > log(1.02) (´expo +´) Figs. 3j, k, l & Fig. 7: 0.019803 (´logisiso´)	0.014889 = log(1.015) (´ logisiso´)	0.014 < log(1.016) (expo -)
Time at inflexion (logistic)	none or 150	150	none
Maximum number of whorls	8	9	6
Number of iterations	280	320	400

Appendix D: Flow chart describing the growth vector model implementation

Initial conditions:

Define growth vector map

U1 (1:3,1:n): position vector in 3D global Cartesian space of n points of the generating curve at time 1

U2 (1:3,1:n): position vector in 3D global Cartesian space of n points of the generating curve at time 2

U1 and U2 are placed in (or near) a X-Z plane

G0 (1:3,1:n): growth vector map

G0 (1:3, 1:n) = U2 (1:3,1:n) - U1 (1:3,1:n)

Define local reference axes

AX : local reference axis parallel to X in global Cartesian coordinates defined by the indices on U of two points located on the left and right of generating curve viewed in X-Z plane

AZ : local reference axis parallel to Z in global Cartesian coordinates defined by the indices on U of two points located on the top and bottom of generating curve viewed in X-Z plane

Define growth curve

dSize(1:tend): instantaneous growth rate of the shell

Compute model:

Starting conditions

for t = 1

Set aperture size
Uold (1:3,1:n) = dSize(1) * U1 (1:3,1:n)

Set growth vector map size for next growth step
Gnew (1:3,1:n) = dSize(1) * G0 (1:3,1:n)

Rotation matrix for next growth step
AX (1:3) = U1 (1:3, Left) - U1 (1:3, Right)
AZ (1:3) = U1 (1:3, Top) - U1 (1:3, Bottom)
AY (1:3) = cross(AZ, AX)
MatRot (1:3, 1:3) = [AX / norm(AX) AY / (norm(AY) AZ / norm(AZ)]

Save Uold

end

Growth vector model

for t = 2:tend

Compute current aperture position
Unew = Uold + MatRot * Gnew
Uold = Unew

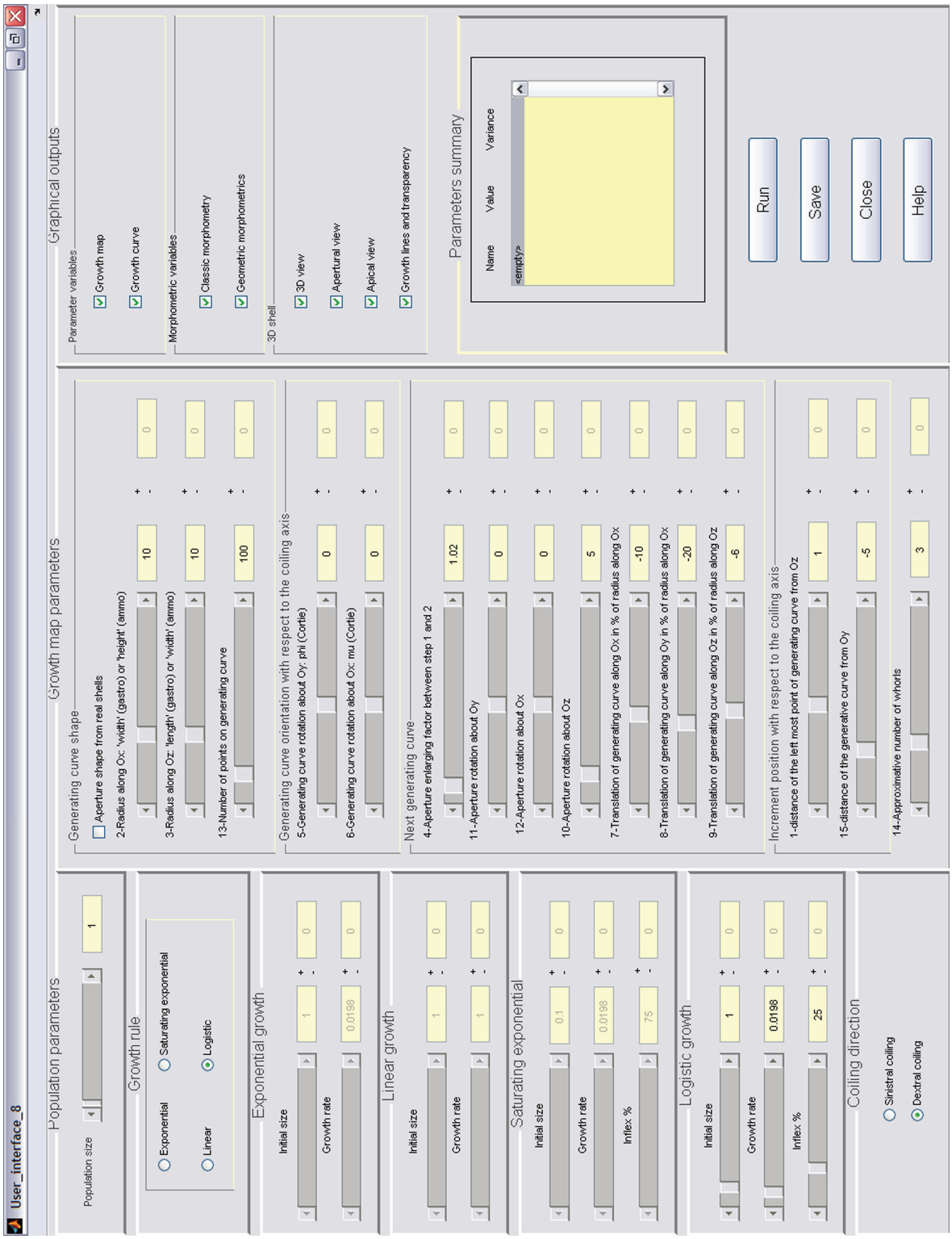
Set growth vector map size
Gnew(1:3,1:n) = dSize (t) * G0(1:3,1:n)

Set rotation matrix for next growth step
AX = Unew (1:3, Left) – Unew (1:3,Right)
AZ = Unew (1:3, Top) - Unew (1:3,Bottom)
AY = cross(AZ, AX)
MatRot (1:3, 1:3) = [AX / norm(AX) AY / (norm(AY) AZ / norm(AZ)]

Save Uold

end

Appendix E: User interface



Chapter 4 - Growth dependent phenotypic variation of molluscan shells: a theoretical and empirical comparison using gastropods

Reference: Urdy, S., Goudemand, N., Bucher, H. & Chirat, R. Growth dependent phenotypic variation of molluscan shells: implications for allometric data interpretation. *Journal of Experimental Zoology Part B*. accepted.

Abstract

In recent years, developmental plasticity has received increasing attention. Particularly, some studies highlighted a possible association between shell shape and growth rates in intertidal gastropods. We use a growth vector model to study how hypothetical growth processes could underlie developmental plasticity in molluscs. The model illustrates that variation in instantaneous shell growth rate (length of incremental growth vectors) can induce variability in allometric curves. Consequently, morphological variation is time-dependent. Basing our model parameters on a study documenting the results of transplants experiments of three gastropods ecomorphs, we reproduce the main aspects of the variation in size, shape and growth rates among populations when bred in their own habitat or transplanted to another's ecotype habitat. In agreement with empirical results, our simulation shows that a flatter growth profile corresponds to conditions of rapid growth. Randomization of time of measurements simulates populations composed of variably aged specimens, thus generating empirical-like growth data. It allows one to investigate how mixing of different 'age classes' can impinge on the variation observed in a population. The model also allows the comparison of allometric slopes using different sub-data sets that correspond to different levels of comparisons (static and ontogenetic allometry). Our model highlights that depending on sub-data sets, the 'main effects' could be attributed to source popualtion or environment. Also, convergence or divergence of allometric slopes is observed depending on the sub-datasets. Although there is evidence that shell shape in gastropods is to some extent growth rate dependent, gaining a general overview of the issue is challenging, in particular because of the scarcity of studies referring to allometry. We argue that the dynamics of development at the 'phenotypic level' constitute a non-reducible level of investigation if one seeks to relate the observed amount of phenotypic variation to variability in the underlying factors.

Key words: molluscs – growth – allometry – morphogenetic model – variation – plasticity.

Contents

I. Introduction

Mollusc ecomorphs have become something of an icon for Neo-Darwinian studies giving rise to a colossal set of reported correlations between shell shapes and some environmental aspects (e.g. wave exposure, shore level, predation, population density…). Because of their extensive phenotypic variation, intertidal gastropods have often been studied in this context. For instance, many studies suggested that variation in shell shape was dependent on a geographical gradient of wave exposure. A relatively large aperture (and large foot) was suggested to favour the ability of snails to clamp on the substrate in wave exposed environments (e.g. Kitching, Muntz & Ebling, 1966; McNair *et al.*, 1981; Crothers, 1983; Paul, 1991) while a relatively elongated shape and narrow aperture were supposed to favour resistance to desiccation during emersions in sheltered environments (e.g. Grahame, Mill & Brown, 1990). Also, in sheltered shores, shells can be relatively thicker than in exposed shores and it is viewed as favouring protection from crabs (e.g. Kitching & Lockwood, 1974).

Sometimes, distinct morphs are found at different heights on the shore (Chapman, 1995; Johannesson, Rolán-Alvarez, Erlandsson, 1997). A well known example is that of the two ecotypes of *Littorina saxatilis* along Galician coasts: the large banded and ridged morphs are preferably found in upper shores where they are subject to frequent emersion whereas the small smooth and unbanded morphs are found in wave exposed lower shores (e.g. Carballo, Caballero & Rolan-Alvarez, 2005). Also, in populations of *Littorina saxatilis* from the West coast of Sweden, morphs in exposed shores tend to be smaller, to have a greater relative aperture size and to have a lower spire than morphs in sheltered shores (Janson, 1982; Johannesson & Johannesson, 1996). A similar pattern is observed for *Littorina unifasciata* from Australian coasts (Chapman, 1995).

The pattern of variation of shell shape of these littorines has been claimed to be largely associated with habitat, perhaps reflecting differential mortality. However, few studies attempted at investigating whether shape differences could cause differential mortality of the different ecotypes (e.g. Janson, 1983). Also, some authors reported that variation around the ecophenotypic trends could be considerable. For instance, Chapman (1997, p. 512) writes that *"[d]ifferences in shape and weight of the shell among shores were not clearly correlated with wave exposure; large and small snails from the same habitats did not show the same patterns from shore to shore (Chapman, 1995). There was a general trend for L. unifasciata to be more elongate with a smaller aperture high on the shore compared to midshore levels on all shores, but there was also considerable small-scale variability in shell morphology among replicate sites at the same level on the shore"*.

Moreover, various transplant and growth experiments in field and laboratory pointed out that natural selection could not be the only factor responsible for the reported correlations between some shell traits and some environmental aspects. Phenotypic plasticity, defined as the capacity of organisms to alter their morphological and life-history traits (e.g. growth rates) in response to their living conditions, slowly emerged as a concept which could account for much of the patterns of variation among mollusc ecomorphs, populations and species (e.g. Palmer, 1992). Interestingly, several studies suggested a relationship between growth rates and shell shape (e.g. Vermeij, 1980; Kemp & Bertness, 1984; Boulding & Hay, 1993).

In *Littorina littorea* species from Southern New England, Kemp & Bertness (1984) report that the slope of ontogenetic allometry[1] of shell length *versus* shell width depends on the density of the snails in the breeding environment and its associated change in growth rates. In naturally high density populations (e.g. superior to 350 snails/m^2), *L. littorea* shells tend to be elongated (shell length exceeds shell width). In low density populations (e.g. inferior to 25 snails/m^2), *L. littorea* shells tend to be globular (shell width approaches shell length). After having decreased the density of a population of *L. littorea* in the field, Kemp & Bertness (1984) report that snails bred at this low density for 12 weeks significantly grew faster[2] than control snails bred at high density during the same period. Also, snails from the low density treatment acquired a rounded aperture[3], became more globular and exhibited a thinner shell with a higher proportion of organic matrix than control snails of similar length. Similarly, Boulding, Buckland-Nicks & Van Alstyne (1993) report that in *Littorina sitkana* from North-Eastern Pacific, the faster growing snails tended to be more globular (lower spire) than the slowly growing snails. Some other authors also discussed an effect of growth rate on shell elongation in *Littorina unifasciata* from Australian coasts. For instance, Chapman (1997) reports that high shore snails grow at a lower rate and are relatively more elongate than snails at the mid-shore levels.

Boulding & Hay (1993) describe a different pattern of variation of shell shape in *Littorina* sp. from North-Eastern Pacific bred under laboratory conditions. Snails bred at low density (about 400 snails/m^2) tended to be elongated and thin-shelled whereas snails bred at high density (about 1000 snails/m^2) were more globular and thick shelled. A similar association between shell thickness and shell globosity at low growth rates has also been reported for *Littorina saxatilis* from Sweden coasts (Johannesson & Johannesson, 1996).

Growth rates have also been shown to be affected by the presence of predators (Vermeij, 1980; Johannesson, 1986; Appleton & Palmer, 1988; Palmer, 1992; Trussel, 1996; Trussel, 2000; Trussel & Smith, 2000; Langerhans & DeWitt, 2002). A decrease in growth rates, due to the presence of predators or starvation, has been related to an increase in the relative shell thickness and labral tooth development for a given shell length in *Nucella lapillus* from North Atlantic (Kitching, Muntz & Ebling, 1966) and the British Isles (Cowell & Crothers, 1970; Palmer, 1990), in *Nucella lamelosa* from North-Eastern Pacific (Appleton & Palmer, 1988) and in *Littorina obtusata* from New England (Trussel, 2000). This indicates a probable decoupling between shell growth and soft tissue growth. It is likely that shell material goes on being secreted while body growth decreases or stops (thus leading to a relative increase in shell thickness, Kitching & Lockwood, 1974; Hutchinson, 1990). In the freshwater gastropod *Physella virgata* bred in laboratory in the presence of predators, a decrease in growth rates has been associated with the development of a rotund shell (Langerhans & DeWitt, 2002).

1 Note that *ontogenetic allometry* can be *longitudinal* or *cross-sectional*. *Longitudinal data* corresponds to the multiple measurements of the same individual at different ages while *cross-sectional data* refers to the measurements of several individuals of different size/age (each individual is measured at a single 'ontogenetic stage' or age). Kemp & Bertness (1984) used longitudinal data (snails were individually marked and measured on a monthly basis during 3 months); however, these 'individual data' have been pooled for each population, so that at the end, their allometric regressions represent a composite of many individuals from a population (cross-sectional data).

2 In lip expansion, shell length and shell weight.

3 Kemp & Bertness (1984) report that the aperture shape ratio (maximum aperture dimension/minimum aperture dimension) was 0.80 ± 0.05 against 0.73 ± 0.05 for snails bred at low and high density respectively.

Ornamentation has also been suggested to be dependent on growth rates. For instance, Boulding, Buckland-Nicks & Van Alstyne (1993) notify that specimens of *Littorina sitkana* grown at high density (low growth rate) were more likely to be ridged than specimens grown at sparse density. Striking changes in ornamentation have been reported for two distinct morphs of *Nodilittorina australis* when transplanted from sloped to vertical rocky shores and *vice versa* (Yeap, Black & Johnson, 2001). In the field, the nodulose morph is associated with low growth rates and sloped rocky shores, whereas the striate morph is associated with high growth rates and vertical rocky shores. Yeap, Black & Johnson (2001) report that even large snails, when transplanted to another habitat, can abruptly alter the sculpture of the newly secreted shell. However, they noticed an asymmetry in the proportion of snails changing their sculpture in the alternative environment. Naturally, the nodulose morphs are more likely to become striated during ontogeny. Accordingly, the transplants experiments resulted in a higher proportion of initially nodulose snails becoming striate when transplanted to vertical shores than the reverse.

Interestingly, some studies investigated the relationships between shell morphology changes and parasitic infections in freshwater snails (Hay, Fredensborg & Poulin, 2005 in *Zeacumantus subcarinatus*; Levri, Dillard & Martin, 2005 in *Potamopyrgus antipodarum*; Zbikowaska, 2003 and Zbikowaska & Zbikowski, 2005 in *Lymnaea stagnalis*) and marine snails (McCarthy, Fitzpatrick & Irwin, 2004 in *Littorina saxatilis*). Diverse authors reported that infection by some of these trematodes could affect snail growth (e.g. Krist & Lively, 1998; Mouritsen, Gorbushin & Jensen, 1999). McCarthy, Fitzpatrick & Irwin (2004) report that shell morphology in *Littorina saxatilis* is altered in infected snails collected in the field, their shell being longer and narrower (higher spire) compared to uninfected snails. Although the latter authors provide no information on growth rates, their observations interestingly parallel the results reported by Johannesson & Johannesson (1996) on the same species.

Although the mentioned studies point out that shell shape in intertidal gastropods is to some extent growth rate dependent, gaining a general overview of the issue is challenging. In particular, in most studies, it is not clear whether *"there is allometry that could result in shape differences among different populations solely due to differences in mean size"* (Boulding, Buckland-Nicks & Van Alstyne, 1993, p. 61). The scarcity of studies describing allometry among populations (or experimental treatments) or referring to ontogenetic data (rather than initial and final states) does not allow a clear empirical confirmation that the reported variation in shell shape does not result from variation in size along a common allometric relationship. Also, it is not clear whether the reported associations between change in growth rates and change in shape are causally related. Although a causal relationship between shell thickness and growth rates seems experimentally well supported, it cannot be ruled out that the relationship between shell globosity and growth rates is only coincidental[4]. Moreover, how variation in growth rates could be causally linked to variation in shape remains unexplored from a theoretical (ontogenetic) point of view.

To investigate how hypothetical changes in shell growth rates could impinge on the amount and direction of phenotypic variation, we use a growth vector model that was discussed

4 Note also that the relationship between shell globosity and growth rates does not seem consistent in different species of littorines.

in chapter 3 to illustrate some fundamental geometrical properties of the logarithmic spiral. In the present state, this model assumes constant shell thickness and does not allow a decoupling of soft tissue growth from shell growth. Under these restrictions, it is not possible to simulate the kind of patterns of variation discussed above for most littorines since they obviously imply important changes in shell thickness (e.g. Kemp & Bertness, 1984; Appleton & Palmer, 1988; Boulding & Hay, 1993; Trussel, 2000). However, this model can used to simulate another study which suggested growth rate dependent shell shape variation in the littorine *Bembicium vittatum* (Johnson & Black, 1998). Although Parsons (1997) reports that, in this species, the snails which exhibited the most rapid growth were visibly thinner, Johnson & Black (1998) do not mention it in their study, suggesting that variation in shell thickness can perhaps be viewed as negligible compared to variation in overall shell shape.

Johnson & Black (1998) investigated the relationships between shell shape and growth rate in three populations of *Bembicium vittatum* showing contrasting phenotypes when grown in distinct habitats. They transplanted samples of each population into one another ecotype's habitat and analysed the change in size and change in shape in these samples after a five month growth period. Relying on this study and that of Black, Turner & Johnson (1994), we attempt at simulating phenotypic variation in this species under hypothetical changes in growth conditions.

In the first section, we point out how the parameters of the growth vector model generally affect shell shape. In particular, we emphasize the close relationship between the size and the geometry of growth increments. In a second section, we illustrate how variation in shell growth rate (length of growth vectors) can induce variability in allometric curves, possibly leading to non-linear correlations among traits. From the model, we derive morphometric variables highlighting how the 'observed' phenotypic variation links to the assumed variability in model parameters. In a third section, we discuss this model by applying it to the empirical data set of Johnson & Black (1998). In agreement with empirical results, our simulations show that a flatter growth profile corresponds to conditions of rapid growth. Additionally, randomization of time of measurements simulates populations composed of variably aged specimens, thus generating empirical-like growth data. It allows one to investigate how mixing of different 'age classes' can 'artificially' minimize or maximize differences among populations. The relationship between variation in model parameters, variation in allometry in adults, in juveniles of given or of different ages, and variation in longitudinal ontogenetic allometry is also discussed. Depending on these 'types' of allometry, allometric slopes are separated according to source population or environment. It means that different samplings of the same data set could lead one to very different conclusions.

II. The growth vector model

The model discussed in chapter 3 will be used to simulate variation in populations. In this section, we briefly outline the properties of this model. For a comparative discussion and a comprehensive description of our model, please refer to chapter 3.

(1) Principles

The growth vector model assumes simple addition of growth vectors, which represent mantle growth during arbitrary (but constant) time steps. The first growth increment defines the so-called growth vectors which are assumed to be constant in direction (relative to the last computed aperture position) during a simulation of a shell (ontogeny). Shell morphology is generated by iteratively adding a growth increment onto the last computed aperture position (discrete model). Each growth increment is obtained by uniformly scaling the initial growth vectors according to various growth rate curves that are used to simulate the mantle growth over time. For convenience, we neglect whorl overlap and we assume that shell thickness is constant and uniform. Thus, shell growth and mantle growth are equivalent. This model can be viewed as a generalization of previous molluscan shell models (Okamoto, 1988; Rice, 1998; see Hammer & Bucher, 2005 and chapter 3).

The growth vector map (first growth increment) and temporal evolution of growth rate constitute the inputs of the model (e.g. Figs. 1d-e). The shell geometry and the morphometric variables derived from it (temporal evolutions of total shell surface area, shell length, aperture shape, etc) are the outputs from the model (e.g. Figs. 1a-c, f-g).

The description of the geometry of the first increment requires at least 7 parameters: one three-dimensional rotation (3), one three-dimensional translation (3), and a scaling factor (*scale*). Since the global coordinates of the vectors of the growth vector map will change with successive increments as the aperture is rotating, the orientation of the moving aperture is estimated at each time step and the growth vector map re-oriented accordingly (for more details, please refer to Hammer & Bucher, 2005 or chapter 3).

Shell growth rate is viewed as an instantaneous growth rate, meaning that it is time-dependent. Shell growth rate at time t corresponds to a measure of 'size' of the growth increment that will be added to the shell during a given time step (between t and $t+1$). We take the length of the growth vectors as this measure of size. For convenience, it is preferable that shell growth rate be independent of the first increment size. In all the simulations, shell growth rate at time t_0 (corresponding to the growth increment built between t_0 and t_1) is thus assumed to equal 1. It is as if lengths of all growth vectors had been divided by the lengths of the first growth vectors between t_0 and t_1. Then, shell growth rate corresponds to the uniform magnification of the initial growth vectors during an unspecified time step. This time step is kept constant in all simulations and is assumed to be 1. As an increment is added at each time step, two shells generated during a given time interval are necessarily made of the same number of growth increments. However, depending on the growth rate, the length of growth vectors will vary. Consequently, the curvilinear length of two shells generated during a given time interval[5] (sum of all lengths of growth vectors), as well as other measurements of shell size, will differ.

Some evidence points out that assuming that growth increments are built during equivalent time interval seem to be justified for intertidal gastropods. In particular, it has been shown that micro-growth increments could reflect lunar day increments while distinct bundles of micro-increments could represent fortnight periods (e.g. Schöne *et al*., 2007, see chapter 3).

5 The 'curvilinear' shell length at point P is the total length of the shell along the spiral trajectory of this point.

But of course, assuming that growth increments are built during equal time intervals may not be generally valid. But even if this assumption is unwarranted, it nevertheless facilitates the theoretical comparison of growth rates and shape in different shells (null-hypothesis model).

Since the successive growth increments are obtained by uniformly scaling the initial growth vectors, the pattern of relative growth rates around the aperture (aperture map *sensu* Rice, 1998) always remains constant during the simulation of a shell (ontogeny). To avoid confusion, Hammer and Bucher's 'apertural map' will be subsequently denoted as 'growth vector map' when it refers to vectors (growth increment), and 'aperture map' when it refers to the pattern of relative growth rates around the aperture (norm of growth vectors). Graphical representations of the growth vector map (growth increment) and of the aperture map are to be found in figure 1e. The insert in figure 1e represents the growth increment (growth vector map).The norm of growth vectors (aperture map) is represented as a function of the position of the points on the aperture in figure 1e (starting from the right and running counter-clockwise. The position 0 is thus on the keel of the ammonite-like shell in figure 1).

In chapter 3, we have investigated the relationships between the temporal evolution of our theoretical shell growth rate (e.g. Fig. 1d) and that of the derived variables (e.g. shell length, shell width, shell surface area, e.g. Fig. 1f). In particular, we have shown that if our growth rate curve was an exponential (respectively bell-shaped) curve, the temporal evolution of shell surface area, as well as linear measurements (e.g. shell length, shell width) was an exponential (respectively logistic) curve. In other words, the temporal evolution of these linear variables roughly corresponds to the integration of our theoretical shell growth rate curves (see chapter 3). Hence, we can assume that, reciprocally, if shell linear dimensions are a horizontal asymptotic function of time, as empirical studies tend to demonstrate (see for instance Bretos, 1980; Picken, 1980; Guzman & Rios, 1987; Black, Turner & Johnson, 1994; Florin, Fried & Reddy, 2000; Iijima, 2001; Schöne *et al.*, 2002; Schöne *et al.*, 2007), then should our theoretical shell growth rate be roughly similar to the derivative of these functions, *i.e*. a bell-shaped curve.

In short, our assumptions are:

- Shell grows discretely by secreting one growth increment per constant time interval.
- In one growth increment, the trajectory of any point P on the aperture is a straight line.
- The directions of these straight lines respective to the last computed aperture position remain constant from an increment to the next.
- Shell growth rate is uniform on the whole aperture (it is a scalar depending on time but not on the position of a point P on the aperture).

(2) Effect of growth rate

Isometric growth will refer to the simple logarithmic spiral model. Reciprocally, allometric (or anisometric) growth is any departure from the simple logarithmic spiral model (e.g. change in aperture shape) throughout ontogeny.

Logarithmic spiral growth (the isometric case per definition) would imply that shell increment length is scaled to aperture size. In this case, growth increment length, as well as other linear dimensions, would increase exponentially over time while the rotation angle would remain constant. This implies that our growth rate at successive time points be a geometrical progression[6] whose common ratio is equal to our scaling

6 A geometrical progression corresponds to an exponen-

factor (*scale*, used to describe the first growth increment). Figure 1 illustrates that if the temporal evolution of the growth rate is a geometrical progression whose common ratio is smaller (Expo-, Fig. 1a), respectively greater (Expo+, Fig. 1c) than *scale*, then the shell grows allometrically. In these three examples, the first increment (Fig. 1e) is the same but the exponential growth rate curves (Fig. 1d) have three different common ratios (see appendix A). The resulting shells (Figs. 1a-c corresponding respectively to cases Expo-, Iso and Expo+) have been scaled to the same diameter to emphasize the shape differences. If the common ratio is smaller than *scale* (Expo- case, Fig. 1d, dashed line), then the allometry of the aperture[7] (Figs. 1a, g, dashed line) corresponds to a compression in the direction perpendicular to the mean coiling axis (*i.e.* radially) and the incremental rotation angle decreases over time. Symmetrically, if the common ratio is greater than *scale* (Expo+ case, Fig. 1d, dashed-dot line), the aperture is radially depressed and the incremental rotation angle increases (Figs. 1c, g, dashed-dot line). In both cases aperture allometry approaches a limit (Fig. 1g).

Note that the radial depression or compression of the aperture of the ammonite-like shell does not affect the keel specifically: the ratios of keel height over aperture height and keel width over aperture width remain the same. Yet, the radial compression of the aperture (for instance, Fig. 1a) may give the impression that the keel is less pronounced, like flattened.

(3) Effect of growth curve

Similarly, any growth rate curve departing from the *scale*-based geometrical progression will give rise to allometries. In the case of a growth rate decaying exponentially after some time steps, shell surface area rapidly approaches a horizontal asymptote (see Fig. 2c, after about 260 time steps) as well as aperture allometry (Fig. 2f). Compared to the isometric case (Fig. 2a), the incremental rotation angle quickly decreases, growth increments get smaller and smaller and aperture get dorso-ventrally compressed. In this example, the convexity of the obtained bivalve-like shell (Fig. 2b) is greater than in the isometric case (Fig. 2a). The overall impression is that of a more realistic shell which recalls the changes at maturity observed on real specimens (decrease in Raup's W, dorso-ventral compression of the aperture; e.g. Vermeij, 2002; Ubukata, 2003).

(4) Effect of incremental angle

It can be shown that the aperture allometry increases quadratically with the initial rotation angle (usually θ_z) about the initial 'coiling' axis (usually O_z) in the first growth increment, all else being equal. In particular, no aperture allometry is observed on 'straight growing shells' (perfect orthocones, $\theta_z = 0$). This is illustrated in figure 3 with the simulation of *Patella*-like shells. Whatever the shape the growth rate curve would take (Fig. 3d), the overall shape of the shell in figure 3a would be the same, except for a magnification factor and the spacing between

tial equation of the type: $y = a \exp(\log(b) \times t) = a \times b^t$, where b is a coefficient called the *common ratio*. For a shell to follow a perfect logarithmic spiral, we have shown in chapter 3 that our shell growth rate at time t should be equal to $scale^{(t-1)}$ where *scale* is the ratio of two linear aperture dimensions of the first growth increment G_0. The growth vectors at time t are given by: $G_{t,i,n} = G_{0,i,n} \times scale^{(t-1)}$, where i corresponds to the indices of points on the aperture and n corresponds to any of the three Cartesian coordinates of growth vectors.

7 In figures 1-4, the aperture shape ratio is defined as the ratio between the maximal dimensions of the aperture taken parallel and perpendicular to the 'coiling axis', in the plane of the aperture. The direction of the 'coiling axis' is determined using a modified version of the stereographic projection method proposed by Ackerly (1989). In figure 8, shell shape ratio is defined as the ratio between the maximal dimensions of the shell taken parallel and perpendicular to the 'coiling axis'.

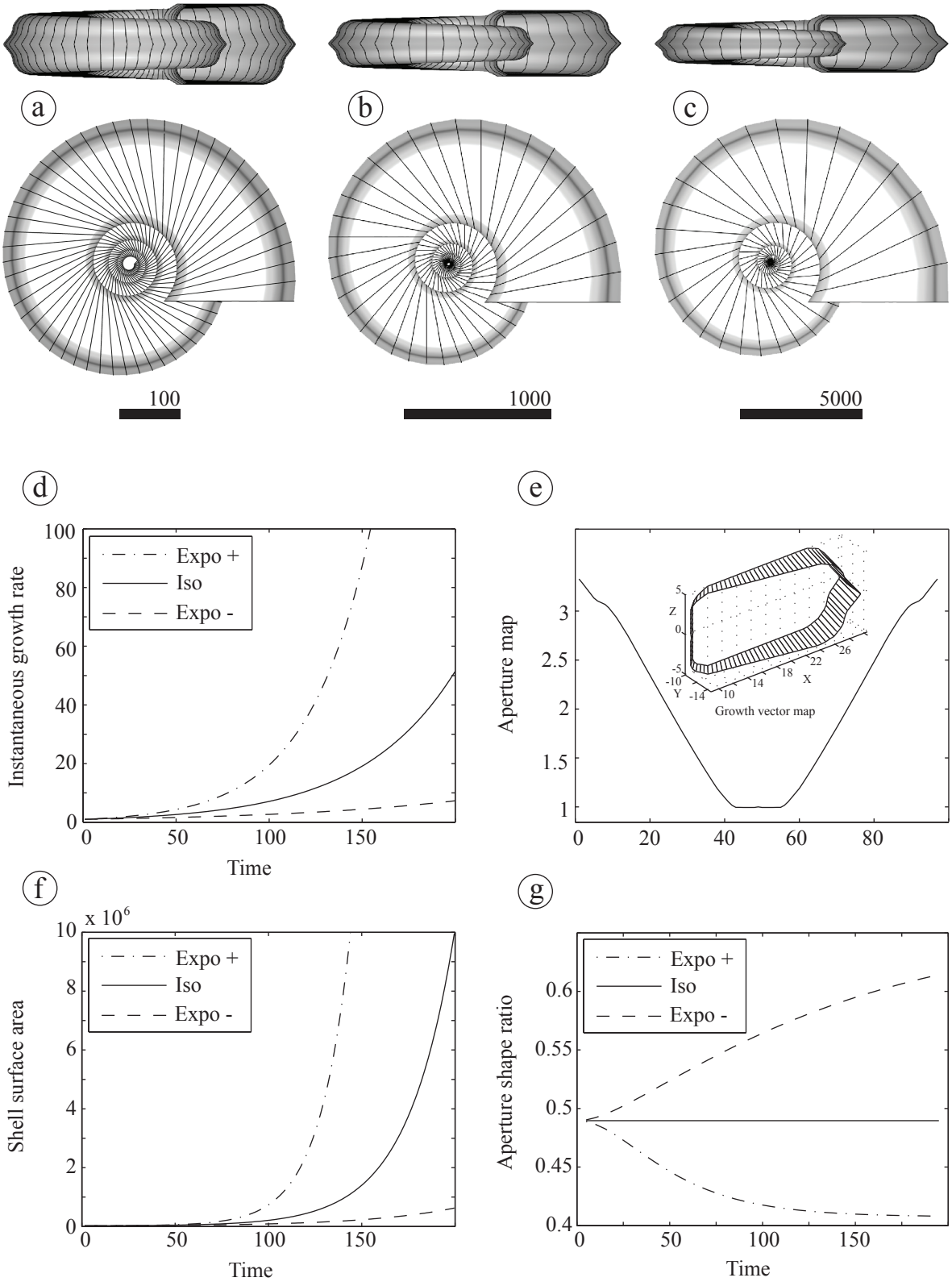


Fig. 1: Effect of growth rate parameter *r* on the allometry of the aperture. **a-c**: shells obtained using a growth rate parameter that is inferior, equal and superior to log (*scale*), respectively. **d-e**: inputs of the model illustrating the growth rate curves (**d**), the aperture map and growth vector map (**e**) used in the simulations. **f-g**: outputs of the model derived from morphometric measurements of the shells in **a-c**. Temporal evolution of shell surface area (**f**) and aperture shape ratio (**g**). Decreasing/increasing the instantaneous growth rate relative to the isometric case (shell **b**) leads to a dorso-ventral compression/depression of the aperture (shell **a, c** respectively).

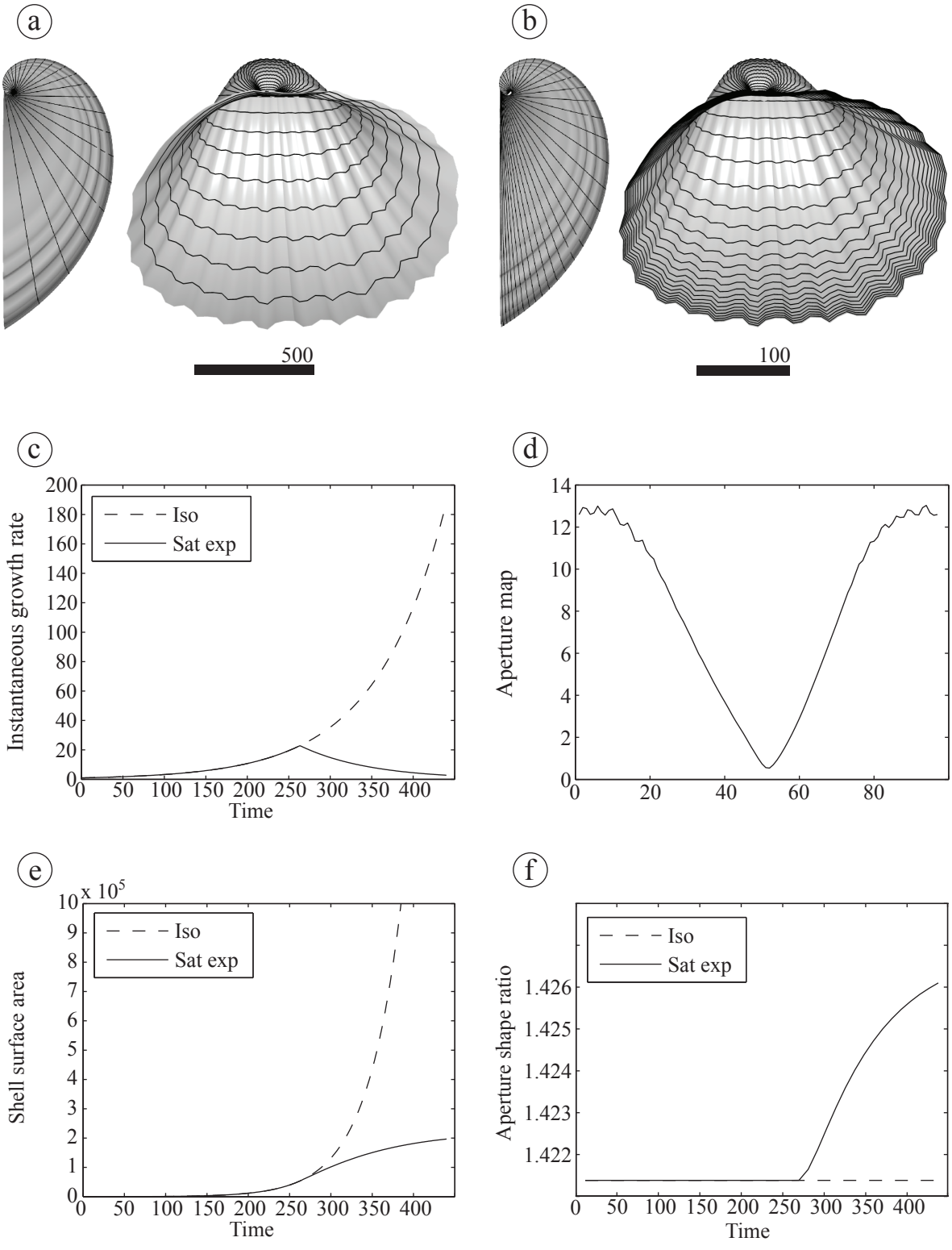


Fig. 2: Effect of growth curves on allometry of the aperture. **a-b**: shells obtained using an exponential growth rate curve those *r* parameter is equal to log(*scale*) (**a**) and a growth rate curve that exponentially decreases after 264 time steps (**b**). **c-d**: inputs of the model illustrating the growth rate curves (**c**), the aperture map (**d**) used in the simulations. **e-f**: outputs of the model derived from morphometric measurements of the shells in **a-b**. Temporal evolution of shell surface area (**e**) and aperture shape ratio (**f**). Decreasing the instantaneous growth rate relative to the isometric case (shell **a**) leads to a dorso-ventral compression in shell **b** after 264 time steps.

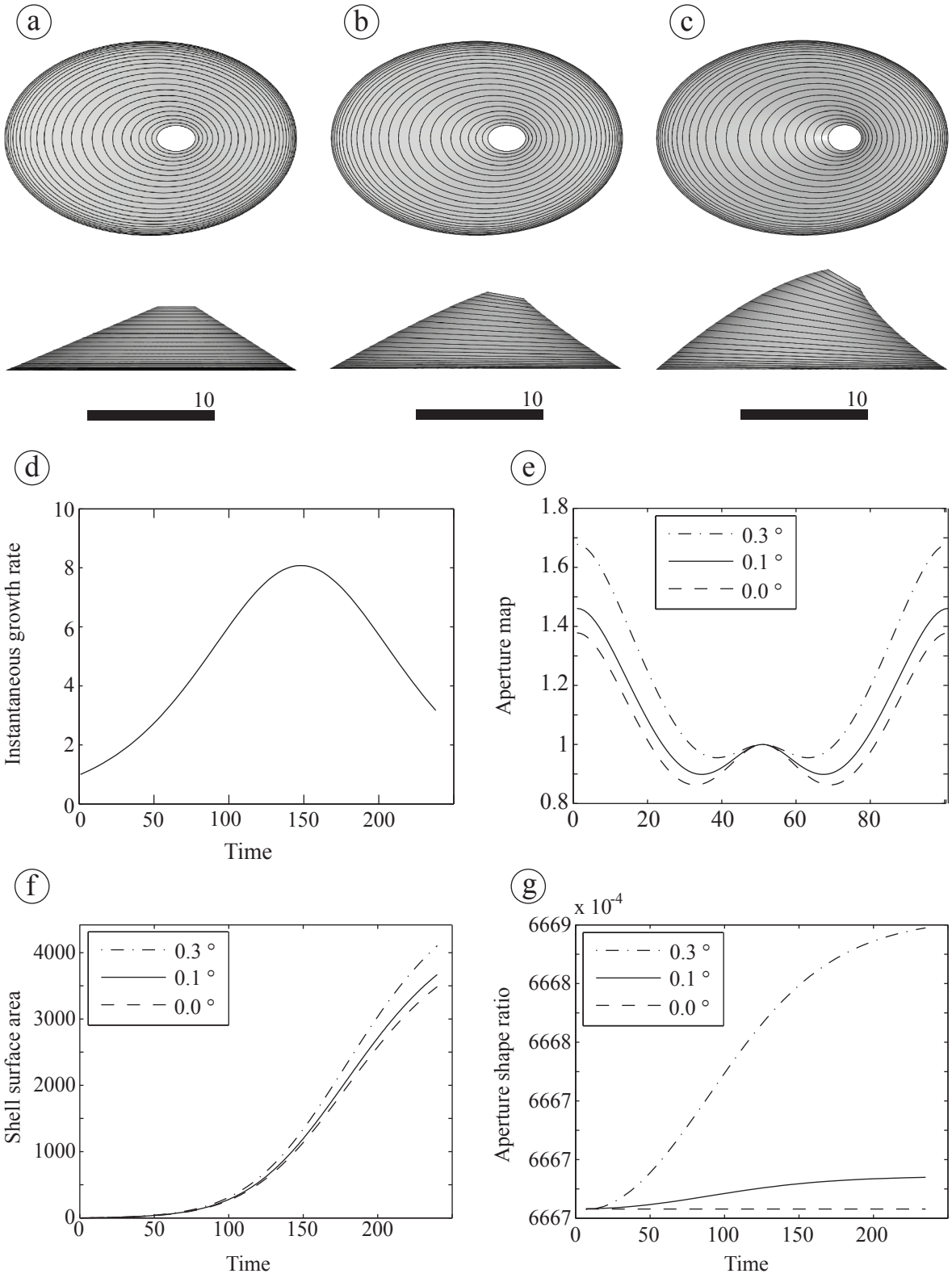


Fig. 3: Effect of the initial incremental angle of rotation (θz) on the allometry of the aperture. **a-c**: shells obtained using a θz that equals 0°, 0.1° and 0.3°, respectively. **d-e**: inputs of the model illustrating the growth rate curve (**d**), and the aperture maps (**e**) used in the simulations. **f-g**: outputs of the model derived from morphometric measurements of the shells in **a-c**. Temporal evolution of shell surface area (**f**) and aperture shape ratio (**g**). Given a growth rate curve that departs from the isometric case, aperture allometry increases quadratically with increasing θz (shells **a** to **c**).

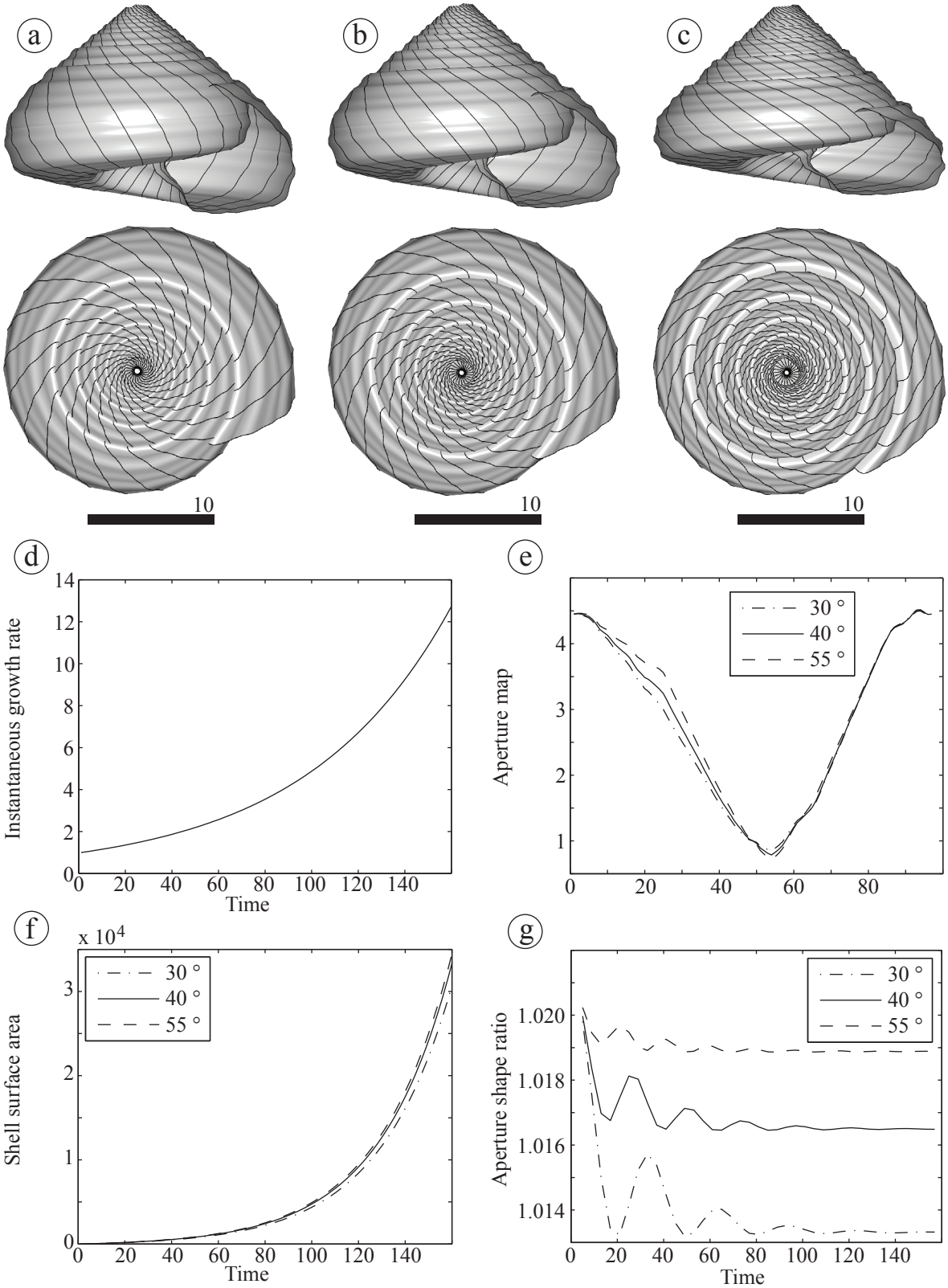


Fig. 4: Effect of aperture inclination (μ) on the allometry of the aperture. **a-c**: shells obtained using a μ that equals 30°, 40° and 55°, respectively. **d-e**: inputs of the model illustrating the growth rate curve (exponential with $r > \log(scale)$ (**d**), and the aperture maps (**e**) used in the simulations. **f-g**: outputs of the model derived from morphometric measurements of the shells in **a-c**. Temporal evolution of shell surface area (**f**) and aperture shape ratio (**g**). Aperture allometry (lateral depression) decreases with increasing μ (shells **a** to **c**). The amplitude of damped oscillations decreases while the frequency increases with increasing μ.

successive apertures. The larger the incremental angle of rotation in the initial growth vector map (Fig. 3e, note the change in aperture map for shells in Figs. 3a-c leading to differences in shell surface area in Fig. 3f), the larger and faster the allometry over time (Fig. 3g, the aperture become compressed in the 'antero-posterior' direction of the *Patella*-like shell, corresponding to a compression from left to right relative to top-bottom direction in the shell of Fig. 3c).

(5) Effect of aperture inclination

The other 'rotation' parameters (μ, θ_x, and θ_y, see chapter 3) also have an effect on the allometry pattern. For instance, the effect of μ, which corresponds to the initial aperture inclination relative to the 'coiling axis', is partly illustrated in figure 4: the temporal evolution of aperture shape ratio shows damped oscillations during ontogeny. Moreover, the larger the initial aperture inclination, the higher the frequency of the damped oscillations and the smaller the resulting allometry for a given growth curve (Expo + here) (Fig. 4). If the aperture is largely inclined relative to the coiling axis, as in the *Trochita*-like shell of figure 4c ($\mu = 55°$), the allometry is barely visible. Note also that the shell in figure 4a is smoother than that in figure 4c: the allometry tend to smooth out the spiral strigations of the aperture. As in the ammonite-like shell (Fig. 1), the spiral strigations scale with the aperture dimensions[8].

8 Note that aperture maps in the three shells of figure 4 slightly change because μ is defined before the translations parameters T_x, T_y, T_z. It can affect the shape of some of the strigations, especially those located to the posterior part of the aperture.

III. Results

(1) Effects of variation in shell growth rate on intra-population phenotypic variation

In this section, we investigate how hypothetical changes in shell growth rate impinge on the amount of observed phenotypic variation. We use a logistic growth curve as defined by Verhulst (1838):

$$dY / dt = r \times Y_{t-1} \times (1 - Y_{t-1} / K) \qquad A.1$$

where Y_t is size at time t; dY/dt is the instantaneous growth rate defined as $(Y_t - Y_{t-1}) / \Delta t$; and K is the asymptotic size (when t approaches infinity).

Discrete integration of A.1 yields:

$$Y_t = r \times Y_{t-1} \times (1 - Y_{t-1} / K + Y_{t-1}) \qquad A.2$$

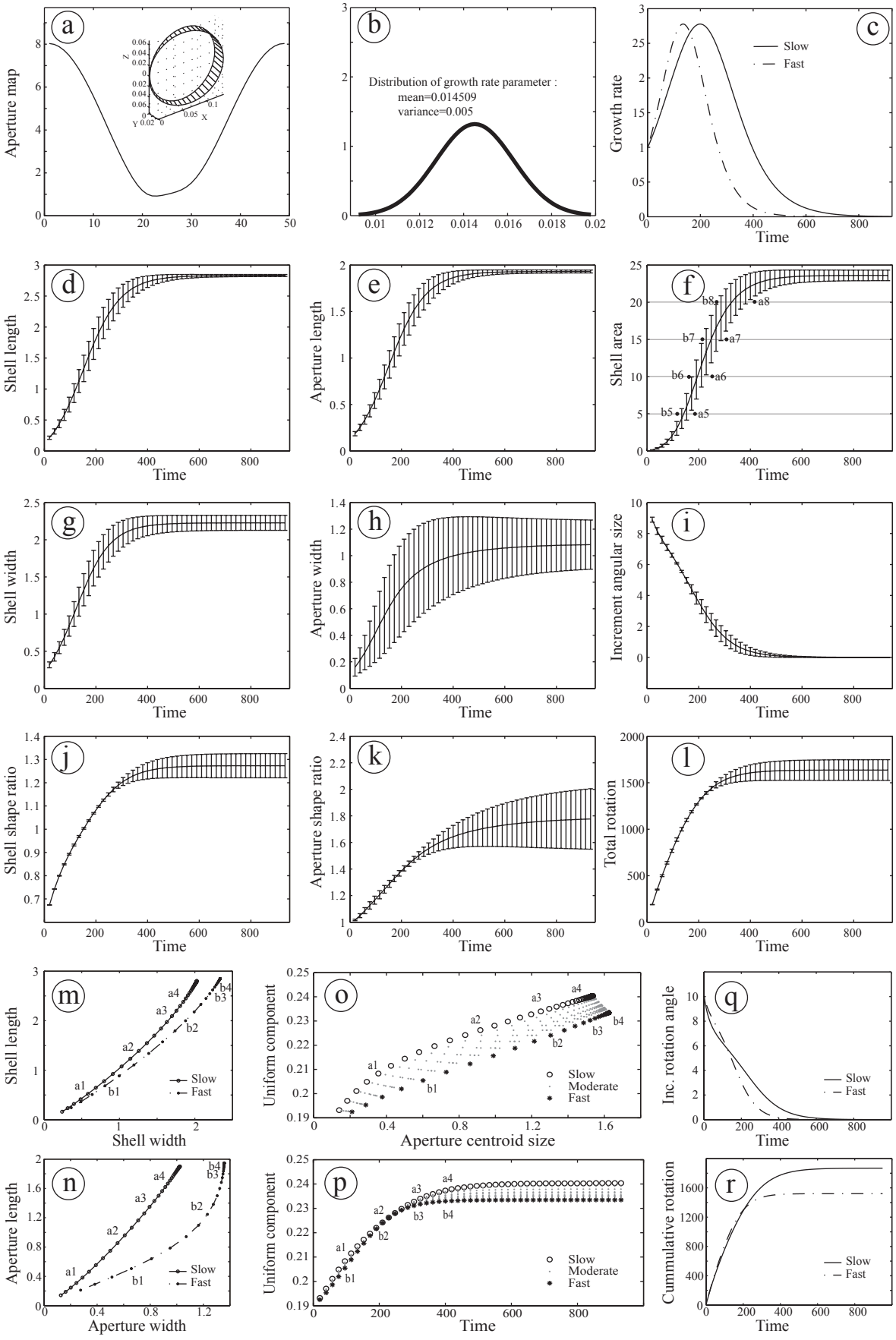
which is equivalent to:

$$Y_t = K \times Y_0 / [(K - Y_0)\, e^{-r\,t} + Y_0] \qquad A.3$$

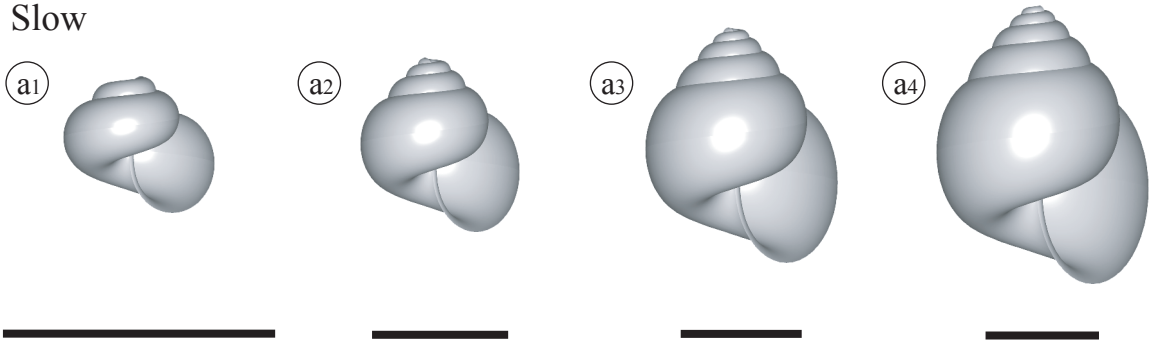
where Y_0 is the initial size (at t_0); and r is the growth rate parameter, also known as the intrinsic growth rate (Tsoularis & Wallace, 2002).

To simulate variation in growth rate in a virtual population, we randomly sample the growth rate parameter r from a normal statistical distribution of given mean and variance (Fig. 5b, mean = log(1.0146) and variance = 0.05). A simulation is run for each of the 25 specimens constituting this virtual population, using the same growth vector map parameters (Fig. 5a) and the same parameters K and Y_0 ($K = 10$ and Y_0

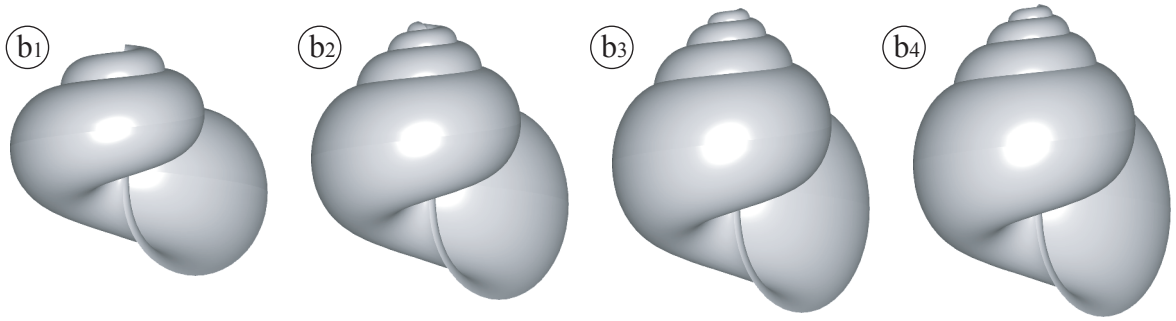
Fig. 5: Effects of variation in shell growth rate on allometric curves and intra-population phenotypic variation given a growth map. **a-c**: inputs of the model. **a**: aperture map and growth vector map (insert). **b**: normal distribution of the growth rate parameter r in a virtual population (25 simulations). **c**: growth rate curves obtained for the two extreme variants. **d-h**: variation in size against time for different measurements. **j, k**: ratios of measurements illustrating shape variation over time. **i, l**: increment angular size and cumulative rotation against time. **q, r**: same graphs for the two extreme variants. **m, n**: classic bivariate allometric plots illustrating allometry in shell shape and aperture shape in the two extreme variants. **o, p**: aperture shape *versus* centroid size and time respectively, using landmarks placed at the aperture.


a
Aperture map
b
Distribution of growth rate parameter :
mean=0.014509
variance=0.005
c
Growth rate
Slow
Fast
Time
d
Shell length
Time
e
Aperture length
Time
f
Shell area
b8
a8
b7
a7
b6
a6
b5
a5
Time
g
Shell width
Time
h
Aperture width
Time
i
Increment angular size
Time
j
Shell shape ratio
Time
k
Aperture shape ratio
Time
l
Total rotation
Time
m
Shell length
Shell width
a1
b1
a2
b2
a3
b3
a4
b4
Slow
Fast
o
Uniform component
Aperture centroid size
Slow
Moderate
Fast
q
Inc. rotation angle
Time
Slow
Fast
n
Aperture length
Aperture width
Slow
Fast
p
Uniform component
Time
Slow
Moderate
Fast
r
Cummulative rotation
Time
Slow
Fast

Slow

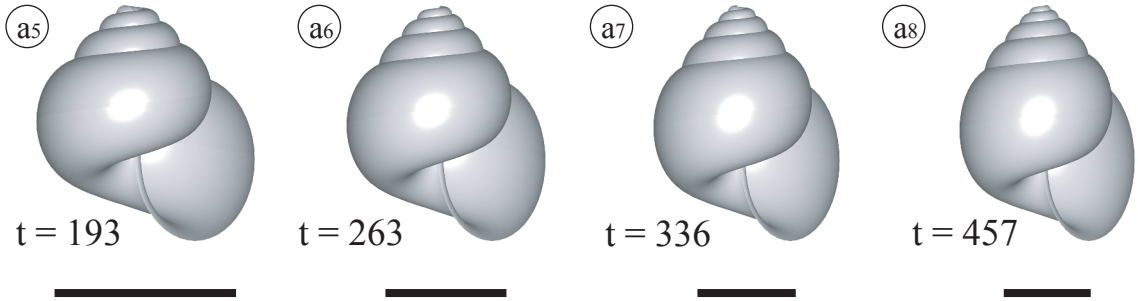


Fast

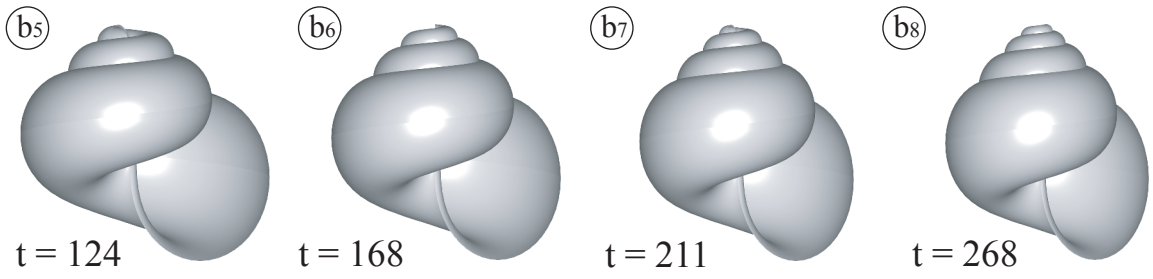


100 increments 200 increments 300 increments 400 increments

Slow



Fast



Shell area = 5 Shell area = 10 Shell area = 15 Shell area = 20

= 1) for all simulations. Figure 5c illustrates the growth rate curves obtained for the most extreme variants (r = 0.0111 and 0.0165). *Specimen a* is described as the slower growing snail (low r, late inflexion point) while *specimen b* is the faster growing snail (large r, early inflexion point). For simplicity, we chose a normal distribution of r that guarantees that all the simulated specimens follow the same ontogenetic allometric trend from a circular aperture toward a radial compression (elongate)[9]. Using four landmarks placed at the aperture every twenty increments, we derive from the model the ontogenetic trajectory of aperture shape for each specimen, as a function of aperture centroid size and time (Figs. 5o, p respectively). The uniform component is computed according to the method described in Rohlf & Bookstein (2003). Classic allometric plots are also presented (Figs. 5m, n). Although all growth rate curves used as inputs (Fig. 5c) were simulated using the same parameter K (asymptotic size), our simulation does lead to small differences in final shell surface areas (Fig. 5f, see also Figs. 5d, e, g, h for linear measurements).

The extreme variants at equal number of increments, corresponding to 100, 200, 300 and 400 time steps are illustrated (Figs. $6a_1$-a_4 /$6b_1$-b_4), see also Fig. 5). The slowly growing variant (Figs. $6a_1$-a_4) gets more rapidly elongated than the rapidly growing variant (Figs. $6b_1$-b_4), with respect to aperture centroid size (Fig. 5o) and time (Fig. 5p). This is also evident from the bivariate allometric plots (Figs. 5m, n). In the first 100 time steps, the incremental rotation angle rapidly decreases for the slowly growing variant, but afterwards it decreases more slowly than for the fast growing variant (Figs. 5q, r). Consequently, until about 200 time steps, the cumulative rotation of the two extreme variants is quite similar, but the slowly growing variant subsequently builds more whorls than the rapidly growing variant (Figs. 5l, $6a_1$-a_4, $6b_1$-b_4; 5 whorls against 4 whorls at 950 time steps). The illustration of the extreme variants at shell surface areas equal to 5, 10, 15 & 20, emphasizes the shape differences among specimens, regardless of differences in size (Figs. $6a_5$-a_8/$6b_5$-b_8).

If the model investigated here reflects reality to some extent, it is clear that variation in a population is expected to be time-dependent (Fig. 5). Note also that maximal variation in linear measurements is expected between 200 and 400 time steps (Figs. 5d-h), whereas ratios of these measurements (shape) exhibit the maximal variation after 400 time steps (Figs. 5j, k). The most extreme differences in aperture shape are found when aperture centroid size is between 0.5 and 1.2 (Fig. 5o), corresponding to 100-300 time steps for the slowly growing variant and 50-200 time steps for the rapidly growing variant. Although this example relies on a null hypothesis model, where the simplest growth rules are

9 The isometric case would be defined as r = log (*scale*), meaning that growth would be isometric at least at the beginning of the simulation and then allometric (radial compression) as the instantaneous growth rate decreases. This case has been referred to as Logisiso in chapter 3. To guarantee that all sampled values of r would be below (respectively above) log(*scale*), we choose a statistical distribution that is to the left (respectively to the right) of the normal distribution of mean log(*scale*), and not overlapping. In the simulated example in figures 5 & 6, *scale* = 1.02, that is r should be equal to 0.0198 for a shell to grow isometrically at the beginning of the simulation.

Fig. 6: Extreme variants observed in the virtual population whose growth rate parameter r is normally distributed and inferior to log(*scale*). **a**: slowly growing specimen (r = 0.0111). **b**: rapidly growing specimen (r = 0.0165). $\mathbf{a_1/b_1}$-$\mathbf{a_4/b_4}$: the two extreme variants at equal numbers of increments. $\mathbf{a_1/b_1}$: 100; $\mathbf{a_2/b_2}$: 200, $\mathbf{a_3/b_3}$: 300 and $\mathbf{a_4/b_4}$: 400 increments. Slowly and rapidly growing pecimens are drawn the same scale to illustrate differences in size. $\mathbf{a_5/b_5}$-$\mathbf{a_8/b_8}$: the same variants at equal shell surface areas. $\mathbf{a_5/b_5}$: 5, $\mathbf{a_6/b_6}$: 10, $\mathbf{a_7/b_7}$: 15 and $\mathbf{a_8/b_8}$: 20 units (see Fig. 5f), corresponding to the time steps indicated next to each shell. The slowly growing variant gets more rapidly elongated than the rapidly growing variant with respect to time ($\mathbf{a_1/b_1}$-$\mathbf{a_4/b_4}$, see also Fig. 5p) and size ($\mathbf{a_5/b_5}$-$\mathbf{a_8/b_8}$, see also Fig. 5o). Note that $\mathbf{a_2/b_2}$ have really similar shapes. Note that the slowing growing variant has more whorls for a given shell area (e.g. $\mathbf{a_8/b_8}$) and for a given number of increments (e.g. $\mathbf{a_4/b_4}$).

assumed and only one parameter is allowed to vary, one would not straightforwardly predict such variation from the model assumptions.

(2) Case study: *Bembicium*

(a) Assumptions

In this section, we apply our model to a particular case study. Johnson & Black (1998) studied three populations of *Bembicium vittatum* from the Abrolhos Islands, Australia. These authors characterized the three populations as being composed of:

- dwarf, highly domed snails from an usually dry tidal pond (P101);
- large, moderately domed snails from a sheltered, regularly inundated pond (P85);
- and relatively flat snails from a vertical exposed shore (S75).

The environments referred to as P101, P85 & S75 by Johnson & Black (1998) will be called here E1, E2 & E3, respectively. Native populations of environments E1, E2 & E3 will be referred to as P1, P2 & P3. Black & Johnson (1998) performed translocation experiments where snails from both ponds (P1, P2) were transplanted to the other pond (E1, E2) and snails from the exposed shore (P3) were transplanted to the sheltered pond (E2). Then, six groups are studied: control snails (P1E1, P2E2, P3E3) and transplanted snails (P1E2, P2E1, P3E2). We run 150 simulations corresponding to 25 individuals per group.

Johnson & Black (1998) point out that snails from P1 tend to mature at a smaller 'size' than snails from P2 and P3 (6 mm against 9 mm in shell width), but it is not known whether snails from P1 mature earlier than snails from P2 and P3. For simplicity, we assume that maturity (here assumed to be equivalent to a null growth rate) is synchronous among populations (first assumption). So, all snails are assumed to achieve their final size in 300 time steps (300 growth increments).

To simulate the differences in morphology reported by Johnson & Black (1998, their figure 1) in the groups P1E1, P2E2 and P3E3, we assume that the parameter *scale* increases from P1 to P3 (1.007, 1.009 & 1.012 respectively, see Table 1. Note that the variation introduced in the parameter *scale* is barely visible in Fig. 8b). It simply means that, for instance, snails from P3 will exhibit a greater whorl expansion rate (*sensu* Raup, 1966) than snails from P2 (all else being equal). In other words, for a given shell length, the snails from P1 will have a smaller aperture than snails from P2. Similarly, snails from P3 will exhibit a larger aperture than snails from P2 for a given shell length. This second assumption relies on the allometric plots of Johnson & Black (1998) which show that regressions of shell length against shell width are differing among P1E1, P2E2 & P3E3: snails from P3 are relatively wider than snails from P2 & P1; snails from P2 are relatively wider than snails from P1. To introduce variation in the parameter *scale* among populations is just one way to reproduce these results qualitatively. Because Johnson & Black (1998) do not provide similar allometric plots for transplanted populations, we assume that the parameter *scale* is not affected by the environment. No intra-group variation in the parameter *scale* is introduced. As a first approximation, this parameter can be thought of as being a characteristic of the source population (P1, P2 or P3), although there is no need to be so (null hypothesis). Other growth vector map parameters are kept constant.

According to the growth curves reported by Black, Turner & Johnson (1994), growth is

asymptotic. We choose a bell-shaped growth rate curve. As no information on the variation in the time of maximal growth rate (at inflexion point) is available, we choose to keep variation in the timing of inflexion as small as possible among and across groups (null hypothesis). To do so, we simulate the growth rate curve as the product of two functions f_1 and f_2:

$$dY / dt = f_1 \times f_2$$

with:

$$f_1(t) = \exp (r \times (t\text{-}1)),$$

being an exponential function whose parameter r, if positive, represents the rate at which $f_1(t)$ increases (Fig. 7a); and

$$f_2(t) = 1 / [1 + \exp (- c_1 \times (t - c_2))],$$

being a sigmoid function bounded between 0 and 1 (Fig. 7b). The sign of the parameter c_1 determines whether the sigmoid is open to the right or to the left (positive or negative c_1 respectively). The value of the parameter c_1 controls for the steepness of the sigmoid function. The parameter c_2 determines the time at inflexion.

Given a positive r, a negative c_1 and a positive c_2, the resulting product of the functions f_1 and f_2 is a bell-shaped curve (Fig. 7c), whose maximum occurs a bit earlier than the inflexion point of f_2. Unlike the Verhulst's equation used in the previous section, this growth rate curve is not symmetric to the right and to the left of the inflexion point. The particular parameterization of this growth rate curve has been used to minimize variation in the timing of maximal growth rate (third assumption, see Fig. 8a, variation in amplitude of growth rate but about same time location of maximal growth rate) and to ensure that all simulated snails would attain a null growth rate at the end of the simulations (300 time steps). The parameters c_1 and c_2 are thus kept constant over all simulations (c_1 = -0.045 & c_2 = 200). This leads to a different pattern of variation in growth curves that the one investigated in the previous section, where variation in growth rate parameter r was linked to variation in the time location of its maximum, but not in its maximal amplitude (Fig. 5). The time of maximal growth rate is checked to be almost the same for all simulations. It is situated at about 178 time steps (mean values: P3E2 = 182 ± 2; P2E2 = 180 ± 2; P1E2 = 179 ± 3; P3E3 = 177 ± 2; P2E1 = 175 ± 3; P1E1 = 174 ± 2).

The growth rate parameter r is assumed to follow a normal distribution of given mean and variance in each of the 6 groups. The mean r in a group is set to be superior to the corresponding log(*scale*), to generate a non-linearity in ontogenetic trajectories that can mimic the domed shape of *Bembicium* (fourth assumption). A bell-shaped growth rate curve which starts at values superior to that expected for isometric growth will lead to a biphasic allometric pattern: at the beginning of the ontogeny, aperture width will increase faster than aperture length, but after the inflexion point, aperture length will increase

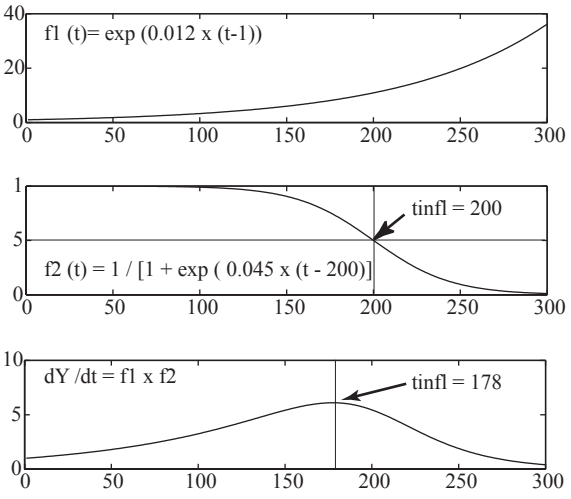


Fig. 7: A growth rate curve is simulated as the product of two functions f_1 and f_2. **a**: f_1. **b**: f_2. **c**: the product of f_1 and f_2, used as input in subsequent simulations. Only the parameter r is allowed to vary in the groups.

faster than aperture width[10]. Few data actually exist to confirm or reject this assumption since allometry of aperture shape is rarely recorded. Nevertheless, Parsons (1997) illustrates, in *Bembicium vittatum*, an allometric plot of shell length against shell width that illustrates a curved pattern on log-log scale in this species (her figure 3). This observation was not reported by the author, but it suggests that snails smaller than 5 mm in shell width could increase their width relatively faster than larger snails (lower allometric slope in juveniles). Our fourth assumption can qualitatively reproduce this pattern.

Johnson & Black (1998) report that growth rate is maximal in the wet pond, corresponding to E2, where transplanted snails (P1E2, P3E2) grow faster (according to all the variables measured) than in their native habitat (P1E1, P3E3). In the dry pond (E1), snails from P2 tend to exhibit a lower growth rate than control snails (P2E2). Consequently, translocation is simulated by increasing the growth rate parameter *r* in populations transplanted to E2 and decreasing it in the population transplanted to E1 (fifth assumption). Before translocation (simulated at 30 time steps), intra-group variation is assumed to be null (and parameters in couplets ″P1E1/P1E2″, ″P2E1/P2E2″ & ″P3E2/P3E3″ are equal; null-hypothesis). Thus, subsequent intra-group variation can fully be attributed to variation in the growth rate parameter *r*.

A final assumption concerns the mean *r* that one has to assign to each of the 6 groups. Johnson & Black (1998, their figures 2 & 3) provide information on growth rates in these groups using the increase in width and amount of rotation (over 5 month growth) against width at the time of translocation (initial width). To relate these measures of growth rates to our theoretical growth rate is not straightforward. From the data provided by Johnson & Black (1998), we can nevertheless 'guess' how the groups should be ranked according to the theoretical parameter *r*. According to the empirical data, the cumulative rotation angle over 5 months at 5 mm width (average initial width) is about:

- 1.75 whorls in P1E2,
- 1.6 whorls in P2E2,
- 1.25 whorls in P3E2,
- 1.1 whorls in P2E1,
- 1 whorl in P1E1,
- and 0.75 whorl in P3E3

As the cumulative rotation over time is an output of our model, we tried different combinations of *r* values (used as inputs) and checked for the ranking of groups according to the cumulative rotation angle. This series of tests was done until the ranking of groups according to the cumulative rotation angle was similar to that obtained by Johnson & Black (1998). A set of mean *r* fulfilling the conditions is: P3E2 > P2E2 > P1E2 > P3E3 > P2E1 > P1E1 (sixth assumption). Within the constraints on the growth rate parameter given by the fourth to sixth assumptions, we arbitrarily choose the mean values of *r* as indicated in Table 1. All groups are assumed to have the same variance of the parameter *r* (0.001 in all cases). Using the means and variance described

	Parameters	**P1**	**P2**	**P3**
E1	*scale*	1.007	1.009	
	r	0.0105	0.011	
	std *r*	0.001	0.001	
E2	*scale*	1.007	1.009	1.012
	r	0.0125	0.013	0.014
	std *r*	0.001	0.001	0.001
E3	*scale*			1.012
	r			0.012
	std *r*			0.001

Table 1: Parameters used to define the groups according to source population and environment.

10 See chapter 3.

above can make the distributions of *r* slightly overlapping. Regardless of the groups, the distribution of *r* is normally distributed (mean = 0.012, standard deviation = 0. 016).

To sum up, our assumptions are:

(1) All snails achieve their final size in the same amount of time (null hypothesis).

(2) The parameter *scale* depends only on the source population (null hypothesis) and its ranking is: P3>P2>P1. No-intra group variation in the parameter *scale* is considered (null-hypothesis).

(3) The growth rate curve is bell-shaped and little variation in inflexion point is allowed (null-hypothesis).

(4) The distribution of *r* in each group does not include the corresponding log(*scale*) (in reference to the isometric case). All randomly sampled *r* values are superior to the corresponding log(*scale*).

(5) The mean growth rate parameter *r* in each group increases when snails are transplanted to E2, and decreases when snails are transplanted to E1. The amount of increase and decrease in the mean growth rate parameter *r* are equal (null hypothesis).

(6) The growth rate parameter *r* in a group follows a normal distribution of given mean and variance. The mean *r* is ranked according to: P3E2 > P2E2 > P1E2 > P3E3 > P2E1 > P1E1. The variance of the growth rate parameter *r* is the same for all groups (null hypothesis).

(b) Size and shape variation

The inputs of the model are illustrated in figures 8a-b. Several outputs are illustrated in figures 8c-f. For instance, note that the mean shell surface area of population P3E3 is slightly above the mean shell area of P1E2 (Fig. 8c), although the mean *r* in P3E3 is inferior to that in P1E2 (Fig. 8a, Table 1). Although P3E3 is assumed to grow more slowly than P1E2 (Fig. 8a), its larger growth increments (larger *scale*) lead to an increase in shell surface area that is faster than in P1E2 (Fig. 8c).

Figure 8d points out that the more domed shells are found in P1E1 while the flatter shells are in P3E2. Figures 9a-f illustrate the mean variants in each of the six groups at the end of the simulations (300 time steps). The two variable parameters act as follow:

- the larger the parameter *scale*, the flatter the shell (from right to left);

- the higher the growth rate parameter *r*, the flatter the shell (from bottom to top).

This is in accordance with the results described by Johnson & Black (1998), who pointed out that a flatter growth profile was associated with an increase in growth rates in E2. These authors measured the shape of the shell after transplantation as the increase in width per whorl over 5 months (hereafter dWi/Wh) against width at the time of translocation (initial width). Similar measurements on the virtual populations are presented in figures 8e-f, with measurements taken between 60 and 150 time steps (one measurement per specimen over this 90 time steps duration[11]). Accordingly, figure 8e illustrates that in E2, all snails tend to exhibit a higher dWi/Wh than in E1 or E3 because of 'environmentally

11 The measurements start at 60 time steps rather than at the time of translocation (30 time steps) because no variation in initial shell width exists at that time (slopes are null). The linear measurements in figures 8e, f have been converted to 'real' millimeters by adopting an *ad hoc* scaling of the measurements on virtual shells. This has been done to facilitate the comparison between the empirical and theoretical study. For instance, Johnson & Black data show that dWi/Wh ranges from 0 to 6.5 mm per whorl for initial shell width between 2.5 and 9.5 mm. Our measurements in figure 8e produce a smaller range of variation in initial width (3.5-4.8 mm) and a smaller range of dWi/Wh (2-6 mm per whorl).Our randomization procedure in figure 8f provided a wider range of variation in initial shell width (2.7-10mm) and in dWi/Wh (1.3-10 mm per whorl) than in figure 8e. Sampling the data over a longer duration, especially one that includes the data after the inflexion point, leads to decrease the range of dWi/Wh, much like Johnson & Black's data. This is explained by the shells becoming more domed after the inflexion point.

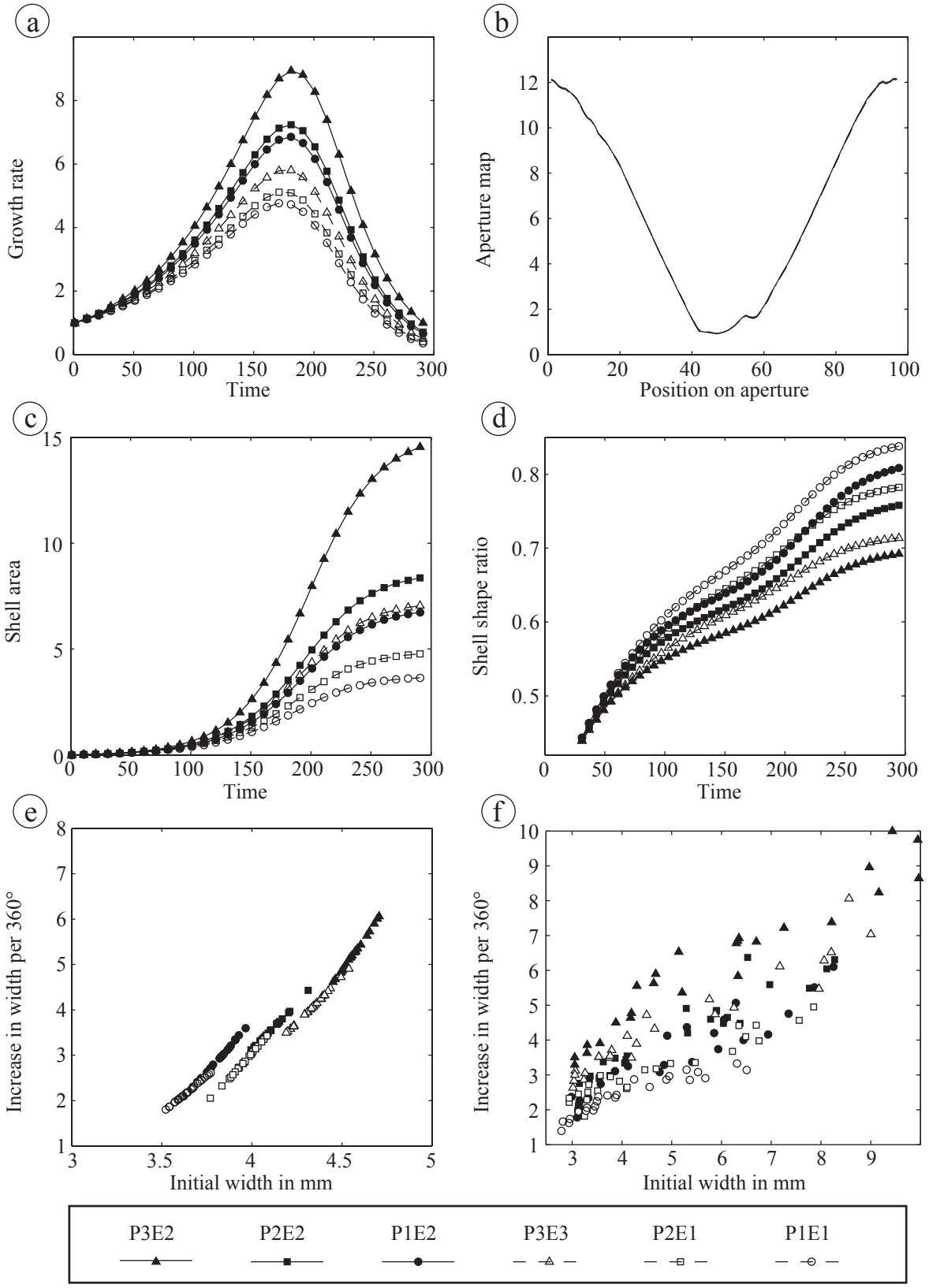


Fig. 8: A simulation of a transplant experiment. **a**: mean growth rate curves used as inputs in each of the six groups. **b**: aperture map in the three native populations. Differences are barely visible. **c**: temporal evolution of mean shell surface area in the six groups. **d**: temporal evolution of shell shape ratio in the six groups. **e**: increase in width per whorl in the six groups against width at the time of translocation. **f**: same variables as in **e**, but with a slight randomization of the time of measurements, simulating the mixing of different 'age classes'.

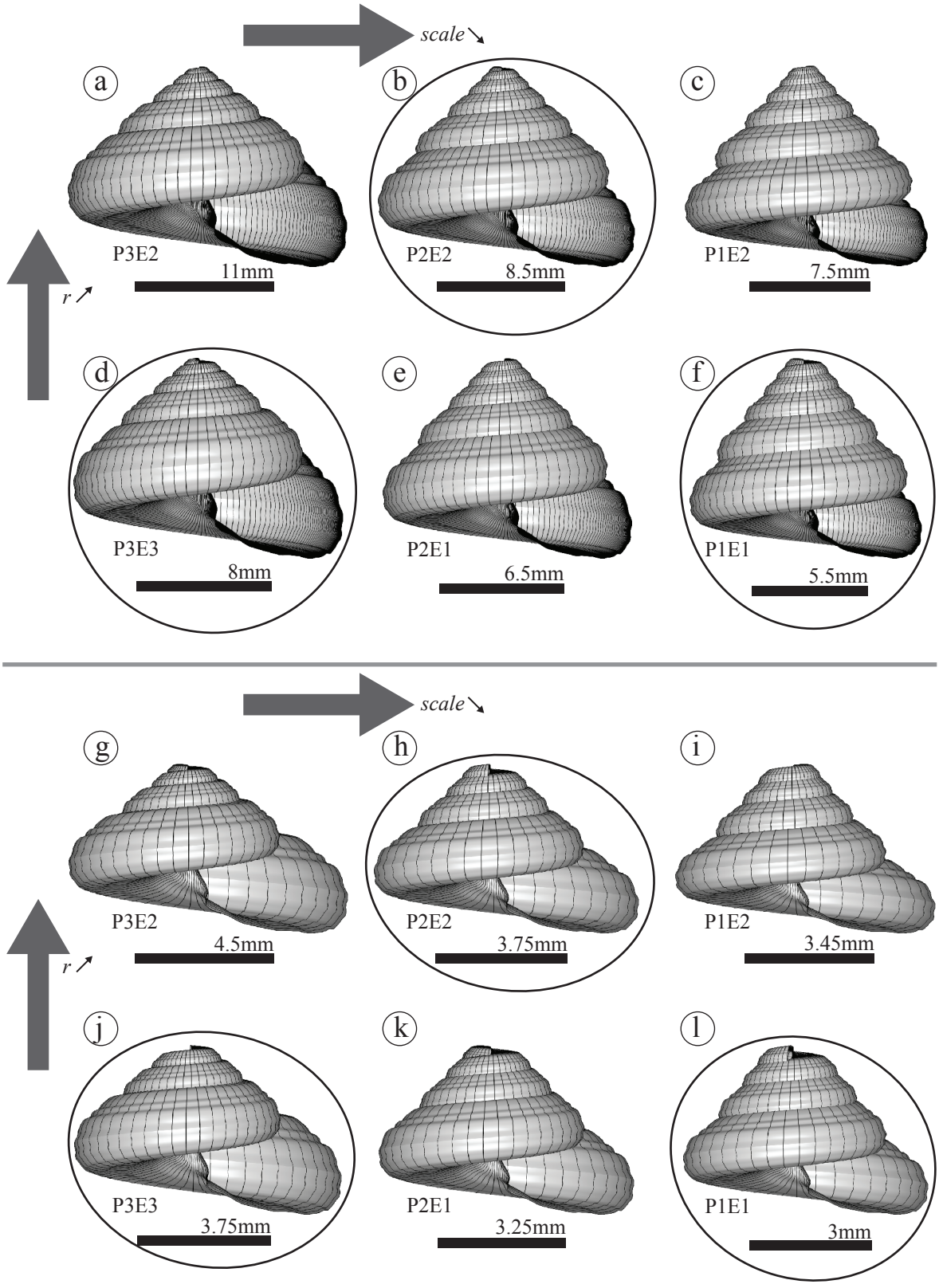


Fig. 9: **a-f**: Mean shell shape in each of the six groups at adulthood (300 time steps), redrawn at the same shell length. **g-l**: Mean shell shape in each of the six groups at a juvenile 'stage' (150 time steps), redrawn at the same shell length. **a/g**: P3E2. **b/h**: P2E2. **c/i**: P1E2. **d/j**: P3E3. **e/k**: P2E1. **f/l**: P1E1. In each panel, **from bottom to top**: the growth rate parameter *r* increases; **from left to right**: the parameter *scale* decreases.

dependent' variation in growth rates (compare white and black symbols). Also, mean dWi/Wh increases from P1 to P3, regardless of environment (variation in *scale*). Juvenile shells (at 150 time steps), corresponding to the mean variants in each group, are illustrated in figures 9 g-l.

More empirical-like data can be generated if one assumes that at the time of translocation snails do not exactly have the same age. A random picking of specimens over a 90 time steps duration with starting values between 30 and 120 time steps[12] was used to obtain the data of figure 8f. Compared with figure 8e, the pattern is obviously noisier. The slopes of the regression lines significantly decrease because of this randomization procedure (see Table 2). Johnson & Black's data (1998, their figure 4) highlight that increase in width per whorl at 5 mm width is about:

- 2 mm per whorl in P1E1
- 2.8 mm per whorl in P2E1
- 3.5 mm per whorl in P1E2 & P3E3
- 3.8 mm per whorl in P2E2
- 4 mm per whorl in P3E2

We obtain about the same ranking of populations using regressions for each population in figure 8f (see Table 2): P1E1 < P2E1 < P1E2 < P2E2 < P3E3 < P3E2.

(c) Static and ontogenetic allometry

The data set generated here can be used to investigate the relationship between variation in the model parameters and variation in the allometric coefficients, using different levels of comparison. How is variability in the model parameters reflected in the allometric coefficients if one compares:

(1) adults or juveniles of a given age?

(2) juveniles of different ages?

(3) the same juveniles at different times of their ontogeny?

The first type of allometry is generally referred to as 'static allometry' since it represents a 'snapshot' of size and shape variation in a population (Cock, 1966; Gould, 1966; Cheverud, 1982). The second and third types are referred to as 'ontogenetic allometry' (Cock, 1966; Gould, 1966; Cheverud, 1982) or 'growth allometry' (Godfrey & Sutherland, 1995) because they represent the ontogenetic change in size and shape in a population. 'Ontogenetic allometry' can be 'cross-sectional' (type 2) or 'longitudinal' (type 3). Longitudinal data corresponds to the multiple measurements of the same individual at different ages while cross-sectional data refers to the measurements of distinct individuals of different size/age (each individual is measured at a single 'ontogenetic stage' or age). In that case, allometry is a composite from many individuals (average trajectory).

The relationship between these different types of allometry remains unclear. Cheverud (1982), quoting Cock (1966) addressed this question: *"[c]an studies of static allometry, in which individuals of one age, typically adults, are studied, be interpreted in terms of relative growth, or is 'the element of true (i.e., ontogenetic) growth…entirely absent from static data' (Cock, 1966, p. 131)?"* Notwithstanding the great number of investigations on allometry, the question of the connection between static and ontogenetic allometry has been rather little studied since then.

Figures 10a-b illustrate the static allometry in adults (300 time steps) and in juveniles (150 time steps) respectively (type 1). The comparison of the regression slopes in each group is

12 ending values between 120 and 210 time steps, respectively.

given in figures 11a, b, respectively[13]. The first observation is that the regression slopes in adults are higher than in juveniles (Figs. 11a, b). This is expected because of the non-linearity in allometric trajectories introduced by the bell-shaped growth rate curve (see also chapter 3). An analysis of covariance points out that all groups have different slopes and different intercepts (Table 2). However, the differences in slopes (and intercepts) are smaller among source populations bred in different environments (e.g. ″P1E1 & P1E2″; ″P2E1 & P2E2″; ″P3E2 & P3E3″) than among different populations bred in common environment (″P1E1 & P2E1″; ″P1E2, P2E2 & P3E2″). Also, regardless of the environment, snails from P1 have the lowest slopes (and highest intercepts); snails from P2 are intermediate and snails from P3 have the highest slopes (and lowest intercepts) (Figs. 11a, b). The lowest intercepts in P3 indicate that these snails are relatively flatter than snails from P1 (P2 are intermediate). This is consistent among the two static allometric plots (Figs. 10a, b; Figs. 11a, b). This can be mainly explained by the variation in the parameter *scale*: the lower the parameter *scale*, the higher the allometric intercept and the more domed the shell (Figs. 11a, b). In both static plots (Figs. 11a, b), the higher slopes in P3 indicate that snails from P3 are those that are the relatively more domed at large sizes, reflecting an interaction between *scale* and *r*.

The parameters *r* and *scale* interact differently in adults and juveniles to determine the allometric slopes. The slopes are decreasing with decreasing *scale* and decreasing *r* in adults (from P3 to P1, Fig. 11a) while they are decreasing with increasing *r* and decreasing *scale* in juveniles (from P3 to P1, Fig. 11b). However, in adults, P3E3 has a slightly higher slope than P3E2, illustrating that in this case an increase in growth rate is linked to a decrease in allometric slopes (Fig. 11a). In same-aged juveniles, the effect of growth rate parameter *r* is more consistent among groups: the higher the *r*, the lower the allometric slope and the flatter the shell (Fig. 11b). This is so because these data on juveniles are taken before the inflexion point, corresponding to the first allometric 'phase' where aperture width increases faster than aperture length (see also Figs. 9g-l for corresponding morphologies).

Ontogenetic cross-sectional data (type 2) were simulated by randomly picking a measurement in each specimen's ontogeny, ensuring that the same specimen was only picked once at a random age (constrained to lie between 60 and 250 time steps). Results are illustrated in figure 10c. The allometric slope of P1E1 is not significantly different from that of P2E1, illustrating a 'convergence' of slopes in these two populations when bred in the same environment (Fig. 11c). Similarly, the allometric slopes of P1E2, P2E2, P3E2 & P3E3 are not significantly different ('convergence'). The effect of variation in growth rate parameter *r* is not always significant: an increase in *r* is related to decrease in allometric slopes in P1 (″P1E1 & P1E2 ″) and P2 (″P2E1 & P2E2″). However, the allometric slopes of P3E2 & P3E3 are not significantly different, though the allometric slope tends to decrease from P3E3 to P3E2 with increasing growth rates (Fig. 11c). Interestingly, the slopes are superior to 1 in ontogenetic allometric plots (Figs. 11c, d) whereas they were inferior to 1 in static allometric plots (Figs. 11a, b). Although the shells in all groups are wider than longer (slope < 1 in static allometric plots), the ontogenetic trend is toward an increase in relative shell length (slope > 1).

13 Allometric coefficients were obtained by linear regression of log-transformed data, a method corresponding to the Huxley-Teissier model discussed in chapter 3. The estimation of coefficients is given in Table 2.

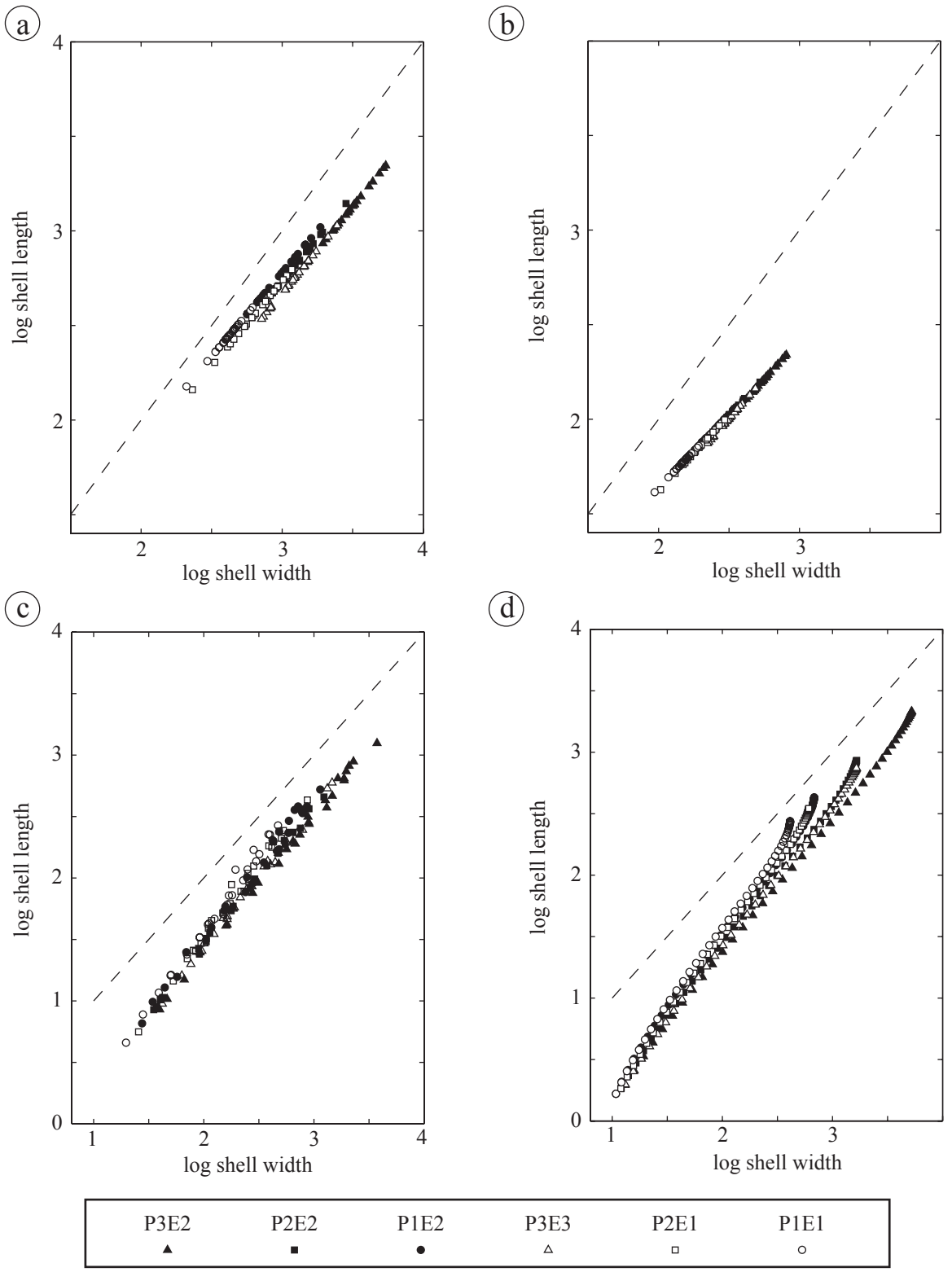


Fig. 10: Classic allometric plots of shell length *versus* shell width using different levels of comparison. **a**: static allometry (adults, at 300 time steps). **b**: static allometry (juveniles, at 150 time steps). **c**: cross-sectional ontogenetic allometry using one measurement per specimen in each group. Measurements are randomly sampled between 60 and 250 time steps. **d**: longitudinal ontogenetic allometry. Each curve represents the ontogeny of mean variants illustrated in Fig. 9. Corresponding regressions of each group in each plot are found in Table 2a-d. The dashed line corresponds to shell width = shell length.

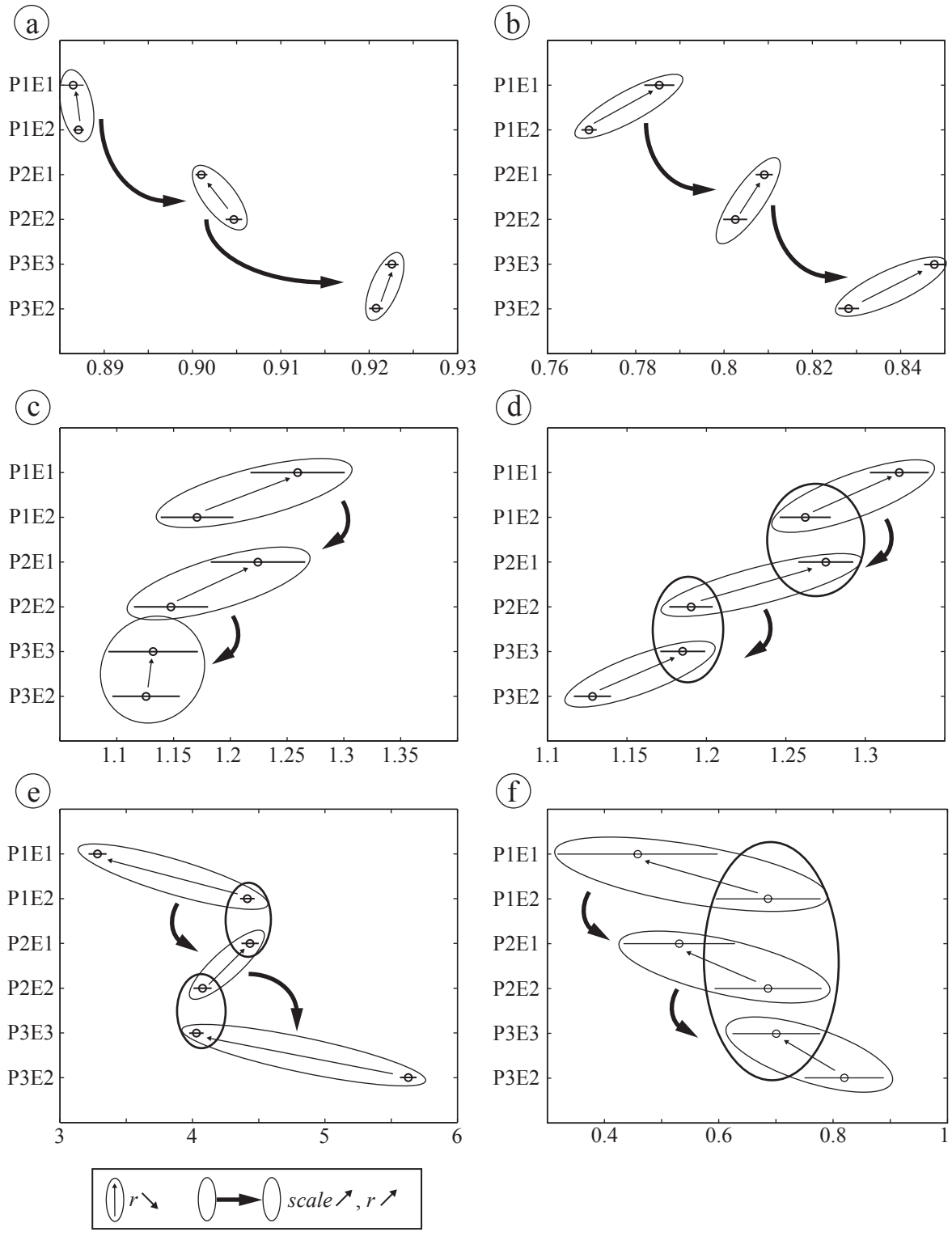


Fig. 11: **a-d**: comparison of slopes in corresponding graphs of Fig. 10. **e-f**: comparison of slopes in corresponding graphs of Figs. 8e-f. Each graph displays an estimation of slopes in each group, as well as the confidence intervals around them. If the confidence intervals overlap, it means that the slopes of the groups being compared are not significantly different (significance level: 5%). **a**: static allometry (adults, at 300 time steps). **b**: static allometry (juveniles, at 150 time steps). **c**: cross-sectional ontogenetic allometry (between 60 and 250 time steps). **d**: longitudinal ontogenetic allometry. Each curve represents the ontogeny of mean variants illustrated in Fig. 9. **e**: increase in width per whorl in the six groups against width at the time of translocation. **f**: same as **e**, with mixed 'aged data' .

Longitudinal ontogenetic allometry (type 3) is illustrated in figure 10d, using the mean variant in each of the six groups. Although cross-sectional data look linear (Fig. 10c), longitudinal data are non-linear. Though, these data have been linearly regressed to allow the comparison with the other types of allometries. Slopes are significantly different among groups (Table 2) and tend to decrease with increasing *r* and with increasing *scale* (Fig. 11d). However, the differences in slopes are larger among source populations bred in different environments than among different populations bred in different environments. For instance, ″P1E1 & P1E2″ (variation in *r*) have more different slopes than ″P1E2 & P2E1″ (or ″P2E2 & P3E3″) (variation in *scale* and *r*). This can lead to 'convergence' in different populations when bred in different environments (″P1E2 & P2E1″; ″P2E2 & P3E3″), and 'divergence' of populations when bred in the same environment (″P2E2 & P3E2″). In other words, snails from P2 and P3 are more similar when they are bred in their native environment than when bred in a common environment (Fig. 11d).

Finally, the slopes of dWi/Wh against initial width (Figs. 8e, f) are compared in figures 11e, f. A faster growth is related to a greater dWi/Wh in 'mixed-age' data (Fig. 11f, flatter shell). However, in figure 11e, the slope of P2E2 is slightly inferior to that of P2E1, illustrating an interaction between *r* and *scale*, that is not visible in figure 11f.

In conclusion, in all allometric plots, the slopes result from the interaction between variation in *scale* and *r.* In static allometric plots, this interaction is different among adults and same-aged juveniles. In both cases, variation in the parameters *scale* and *r* among populations seems to be more important than intra-population variation in the parameter *r*. Comparison among adults hardly reveals anything about the ontogenetic patterns but data on same-aged juveniles is relatively informative. For instance, in same-aged juveniles, the decrease in allometric slopes with increasing growth rates is consistent with ontogenetic allometric plots. It means that a flatter growth profile is mainly associated with an increase in growth rates. Also, the slopes in adults are significantly higher than in same-aged juveniles, reflecting the non-linearity in ontogenetic allometry. However, in ontogenetic allometric plots, convergence of slopes can be observed in groups that are well separated in static allometric plots. Typically, in ontogenetic allometric plots, intra-population variation in the parameter *r* seems more important than variation in the parameters *scale* and *r* among populations. In cross-sectional data, the effect of *r* is marginally significant, due to the increase in variance resulting from the 'averaging' of slightly variable ontogenetic trajectories. In longitudinal ontogenetic allometry, the effect of intra-population variation in growth rates transcends that of variation in the parameters *scale* and *r* among populations, so that different populations in different environments may have ontogenetic patterns that are more similar than the same population in two environments or two populations in the same environment. Also, ontogenetic allometric slopes are superior to 1 indicating that snails tend to become relatively more domed during ontogeny. This pattern is not reflected in static allometric plots that provide only information on variation in shape among groups at one time. In this sense, static allometric slopes do not provide information on change in shape during ontogeny. However, the comparison of several static allometric slopes does provide information on ontogenetic shape changes. Interestingly, a slight

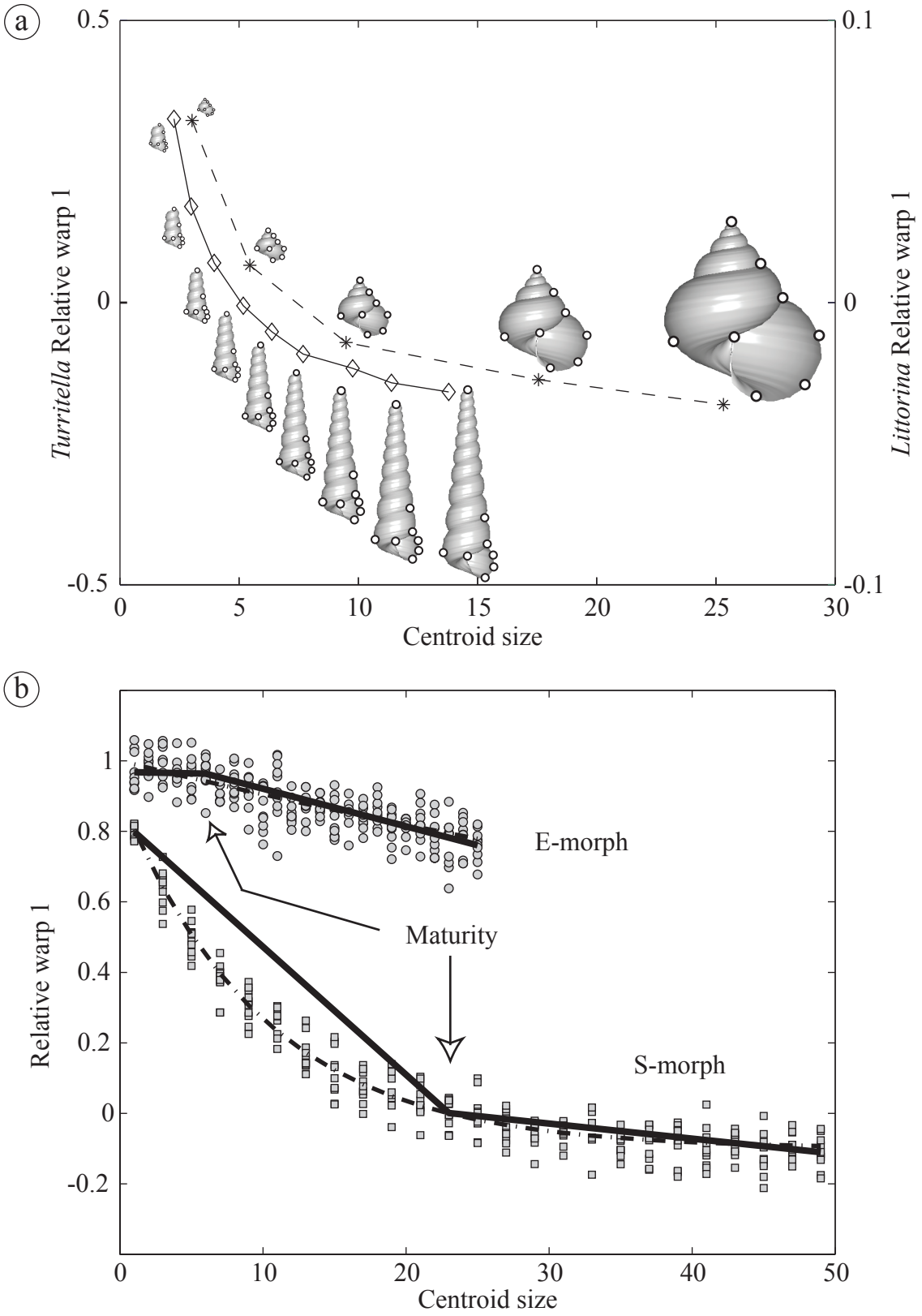
mixing of different aged-specimen can lead to patterns (convergences) that are not found in 'error-free' data.

These theoretical considerations can have several implications for the interpretation of empirical studies. In particular, the range of sizes investigated can have important consequences. For instance, consider that cross-sectional data are like that of figure 10c: the conclusion would be that the populations are 'phenotypically plastic' because variation among environments transcends that among populations. But consider now that the range of cross-sectional data is reduced: the observed pattern would be similar to that of figures 11a or b. The conclusion would be that populations are 'genetically' differentiated because variation among populations transcends that among environments. Interestingly, Parsons (1997) investigated allometric patterns among *Bembicium vittatum* offsprings that were presumably of the same generation, corresponding to a size range of 5-11 mm in shell width. These data are probably more static than that of Johnson & Black (1998) who investigated a larger size range (5-19 mm in shell width). In accordance with our theoretical study, Parsons (1997) observed more variation among populations than among environments, concluding that the plastic component of shell shape was small compared to genetic differentiation among populations. Johnson & Black (1998) concluded to the contrary that the plastic component of shell shape was large compared to the genetic differentiation among populations. Accordingly, the allometric slopes of Parsons (1997) are lower than that of Johnson & Black (1998) (1.151-1.489 against 1.396-1.875), possibly pointing out that the data of Parsons (1997) are more static ('same aged specimens'). Note also, that over the size range investigated by Parsons (1997), the allometric regression of Johnson & Black (1998) in P2E2 would not be really different from that of P3E3. Then, it cannot be ruled out that some of the contrasts between these conclusions come from the different types of data investigated.

IV. Discussion

Our model illustrates how variation in growth rates could impinge on allometry and variation in shape. Although there is evidence that allometries in molluscs are to some extent growth dependent and variable among individuals, populations and species, it remains impossible to gain a general overview of the relationship between growth rate and shape in gastropods, especially because of the scarcity of data referring to allometry. In particular, it is not clear at all from most studies discussed that the shape differentiation between morphs (ecotypes or parasite-infected snails) implies an ontogenetic allometry of aperture shape as in our model. In *Littorina spp*, some variation in shell shape results from differences in aperture shape (Clarke, Grahame & Mill, 1999). Also, Kemp & Bertness's study (1984) suggests that *Littorina littorea* is variable in the roundedness of the aperture (as shown by their data on the aperture index in their table 2, p. 812), thus implying that the allometry they observed between shell width and shell length can be linked to aperture shape changes.

However, although highly frequent, allometries are rarely accounted for in molluscs, making it difficult to compare our results to empirical data. Only in one recent study (Hollander, Adams & Johannesson, 2006), are the ontogenetic allometric patterns the real focus of the study. Unfortunately, this study does not allow us to conclude anything about allometry nor isometry (in an ontogenetic sense) because


a
0.5
0
-0.5
0.1
0
-0.1
Turritella Relative warp 1
Littorina Relative warp 1
0
5
10
15
20
25
30
Centroid size
b
1
0.8
0.6
0.4
0.2
0
-0.2
Relative warp 1
E-morph
Maturity
S-morph
0
10
20
30
40
50
Centroid size

of the landmarks that these authors chose to digitized. Working on *Littorina saxatilis*, an indeterminately growing gastropod, Hollander, Adams & Johannesson (2006) used landmarks similar to those represented in figure 12. Their goal was to characterize the ontogenetic growth pattern of variation between two morphs inhabiting different habitats, between males and females and between two growth stages defined by the onset of maturity. But with these landmarks, it is expected that the first relative warp, representing the size of the aperture relative to whole shell, decreases according to an exponential curve as the number of whorls increases (the shell growing like a logarithmic spiral or not). For instance, a perfectly isometrically growing *Turritella*-like shell (perfect logarithmic spiral, Fig. 12a, diamonds, solid line) can be diagnosed as allometrically growing simply because the first relative warp[14] is significantly correlated with the landmark centroid size ($p<0.01$). Similar measurements on isometric *Littorina*-like shells (Fig. 12a, stars, dotted line) leads to the same conclusion, although this effect is less pronounced (note the scale of the first component on the right-hand side). Shape variation in spire height (conical angle) is expected to introduce confusion in the interpretation of these data since the lower the spire, the flatter the exponential relationship between shape variables and centroid size (compare *Turritella*-like shells and *Littorina*-like shells in Fig. 12a).

From the data of Hollander, Adams & Johannesson (2006), the S-morph matur++es at a larger size than the E-morph (10 mm against 4 mm). These authors also point out that the E-morph exhibits a lower spire than the S-morph. From this, we conclude that the relationship between the first relative warp and centroid size is an exponential that decreases slower in the E-morph case than in the S-morph one (Fig. 12b). Assuming that the size at maturity is occurring at a smaller size in the E-morph than in the S-morph, it can be arbitrarily be concluded that:

- the S-morph grow allometrically as juvenile and isometrically as adult (Fig. 12b)
- the E-morph grow isometrically as juvenile and allometrically as adult (Fig. 12b)

That is the conclusion arrived at by Hollander, Adams & Johannesson (2006). Then, the main results of this study can be strongly suspected to stem from a choice of landmarks that has little relevance to the accretionary growth process on one hand, and from the discretization of allometric pattern according to different sizes at maturity on the other hand. These choices can hardly be rationalized according to the objectives for which this study was undertaken, namely the demonstration of *"the importance of developmental shifts in the evolution of species with indeterminate growth"* (Hollander, Adams & Johannesson, 2006, p. 2496). To demonstrate such shifts in development, landmarks taken at

14 The first relative warp is the unique component in this simple case.

Fig. 12: **a**: Uniform component *versus* centroid size of two isometric shell shapes using landmarks similar to Hollander, Adams & Johannesson (2006). 8 landmarks are sampled every full revolution. **Diamonds and solid line:** *Turittella*-like shape; **Stars and broken line**: *Littorina*-like shape. Note that with such measurements, it is expected that the first relative warp exponentially decreases as the number of whorls increases. Testing the correlation between this shape variable and centroid size would lead to the rejection of the hypothesis according to which both variables are uncorrelated (p-value<0.05). Note that isometric and allometric growth will both generate exponential curves using this choice of landmarks. **b**: First relative warp *versus* centroid size in two morphs differing in spire height. If the **E-morph** has a lower spire than the **S-morph**, it is expected that the curve for the E-morph is a slower decreasing exponential, compared to that of the S-morph. **Grey**: random sampling of 'specimens' around the main trend (black doted line). **Black**: pseudo-allometric plots, as displayed by Hollander, Adams & Johannesson (2006), illustrating the effect of discretization among juveniles and adult stages. In the E-morph this maturity is occurring at a small size (say at centroid size ≈ 5), the juvenile stage can arbitrarily be diagnosed as isometric, whereas the adult stage is diagnosed as allometric. In the S-morph maturity is occurring at a larger size (say at centroid size ≈ 20), the juvenile stage can arbitrarily be diagnosed as allometric, and the adult stage as isometric.

the aperture (or standardization for the variation in number of whorls among specimens), or alternatively, plots of the natural logarithms of linear measurements (shell length *versus* shell width) would have been of less disputable value.

V. Conclusion

Given the scarcity of data referring to allometry, we cannot test our model further. It makes it difficult to assess the patterns of covariation between growth and shape in molluscs for which a large amount of empirical data is potentially already available. Nonetheless, our model has the virtue of allowing the testing of some basic hypotheses of growth. It also sheds light on how 'development' impinges on the observed pattern of variation.

In practice, as in our model, 'genetic' and 'environmental' effects are inseparable. According to the assumptions underlying the simulation of phenotypic variation in *Bembicium*, the *scale* parameter corresponds to 'genetic' differentiation among populations while the growth rate parameter *r* is assumed to be dependent on both population and environment. In the resulting allometric plots, the effects of these two parameters are hardly separable (interaction is marginally significant in cross-sectional data and highly significant in all other cases). We have shown that analysis of different sub-data sets can possibly have important consequences on the observed pattern of variation and its subsequent interpretations. The Neo-Darwininan paradigm requires that one is able to discriminate between the effects of underlying genetic differences (heritable phenotypic variation) and the plastic response to environment. For instance, Johnson & Black (1998, p. 95) write that *"[f]undamental to interpreting the evolutionary significance of this variation is the determination of how much it is a direct, plastic response to local environmental conditions and how much it results from underlying genetic variation"*. Yet, even in theory, using a simple model where we know which parameters are variable and how they vary, it is practically impossible to know how much of the variation results from environmentally-dependent variation in the growth rate parameter *r* and how much of the variation results from characteristics of populations in a given environmemt (*scale* and *r*). Moreover, the connection between variation in model parameters and variation in size and shape can only be appreciated if one considers the dynamics of development at the 'phenotypic level'. Phenotypic variation is generally time-dependent, and this questions the validity of the statistical approach consisting in separating the amount of variation among 'genetic' and 'environmental' components. As discussed by Levins & Lewontin (1980, p. 74),*"[t]he analysis of variance is a tautological partitioning of total variance among observations into main effects and interactions of various orders. Yet, as every professional statistician knows, the partitioning does not separate causes except where there is no interaction… Yet natural and social scientists persist in reifying the main effect and interaction variance that are calculated, converting them into measures of separate causes and static interactions of causes. Moreover, they act as if 'main effects' were really 'main' causes in the every day English meaning of the word and that interactions are really of a second order of importance. Interaction in this view is what is left over after main effects are accounted for. This attitude toward main effects and interactions is a form of the ceteris paribus assumption that plays such a central role in all Cartesian science, but that has become an unconscious part*

of the ideology of the analysis of variance". On conceptual and philosophical grounds, some authors have reinforced the argument of advocating "causal democracy" of genetic and environmental factors on morphology (Oyama, 2000). Our approach provides a practical way to address the relationship between phenotypic variation and the variation in the underlying factors.

VI. References

Ackerly, S. C. (1989). Shell coiling in gastropods: analysis by stereographic projection. *Palaios* **4,** 374-378.

Appleton, R. D. & Palmer, A. R. (1988). Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proceedings of the National Academy of Sciences of the United States of America* **85,** 4387-4391.

Black, R., Turner, S. J. & Johnson, M. S. (1994). The early life history of *Bembicium vittatum* Philippi, 1846 (Gastropoda, Littorinidae). *Veliger* **37,** 393-399.

Boulding, E. G., Buckland-Nicks, J. & Van Alstyne, K. L. (1993). Morphological and allozyme variation in *Littorina sitkana* and related *Littorina* species from the Northeastern Pacific. *Veliger* **36,** 43-68.

Boulding, E. G. & Hay, T. K. (1993). Quantitative genetics of shell form of an intertidal snail: constraints on short-term response to selection. *Evolution* **47,** 576-592.

Bretos, M. (1980). Age-determination in the keyhole Limpet *Fissurella crassa* Lamarck (Archaeogastropoda, Fissurellidae) based on shell growth rings. *Biological Bulletin* **159,** 606-612.

Carballo, M., Caballero, A. & Rolán-Alvarez, E. (2005). Habitat-dependent ecotype micro-distribution at the mid-shore in natural populations of *Littorina saxatilis*. *Hydrobiologia* **548,** 307-311.

Chapman, M. G. (1995). Spatial patterns of shell shape of three species of co-existing littorinid snails in New South Wales, Australia. *Journal of Molluscan Studies* **61,** 141-162.

Chapman, M. G. (1997). Relationships between shell shape, water reserves, survival and growth of highshore littorinids under experimental conditions in New South Wales, Australia. *Journal of Molluscan Studies* **63,** 511-529.

Cheverud, J. M. (1982). Relationships among ontogenetic, static and evolutionary allometry. *American Journal of Physical Anthropology* **59,** 139-149.

Clarke, R. K., Grahame, J. & Mill, P. J. (1999). Variation and constraint in the shells of two sibling species of intertidal rough periwinkles (Gastropoda: *Littorina* spp.). *Journal of Zoology* **247,** 145-154.

Cock, A. (1966). Genetical aspects of metrical growth and form in animals. *Quarterly Review of Biology* **41,** 131-190.

Cowell, E. B. & Crothers, J. H. (1970). Occurrence of multiple rows of teeth in shell of dog-whelk *Nucella lapillus*. *Journal of the Marine Biological Association of the United Kingdom* **50,** 1101-1111.

Crothers, J. H. (1983). Some observations on shell shape variation in North American populations of *Nucella lapillus* (L). *Biological Journal of the Linnean Society* **19,** 237-274.

Florin, L., Fried, B. & Reddy, A. (2000). Growth and fecundity of *Lymnaea elodes* (Gastropoda: Lymnaeidae) under laboratory conditions. *Veliger* **43,** 78-81.

Godfrey, L. R. & Sutherland, M. R. (1995). What's growth got to do with it? Process and product in the evolution of ontogeny. *Journal of Human Evolution* **29,** 405-431.

Gould, S. J. (1966). Allometry and size in ontogeny and phylogeny. *Biological Reviews of the Cambridge Philosophical Society* **41,** 587-640.

Grahame, J., Mill, P. J. & Brown, A. C. (1990). Adaptive and nonadaptive variation in two species of rough periwinkle (*Littorina*) on british shores. *Hydrobiologia* **193,** 223-231.

Guzman, L. F. & Rios, C. F. (1987). Age and growth of the sub-antarctic limpet *Nacella (Patinigera) magellanica magellanica* (Gmelin, 1791) from the Strait of Magellan, Chile. *Veliger* **30,** 159-166.

Hammer, O. & Bucher, H. (2005). Models for the morphogenesis of the molluscan shell. *Lethaia* **38,** 111-122.

Hay, K. B., Fredensborg, B. L. & Poulin, R. (2005). Trematode-induced alterations in shell shape of the mud snail *Zeacumantus subcarinatus* (Prosobranchia: Batillariidae). *Journal of the Marine Biological Association of the United Kingdom* **85,** 989-992.

Hollander, J., Adams, D. C. & Johannesson, K. (2006). Evolution of adaptation through allometric shifts in a marine snail. *Evolution* **60,** 2490-2497.

Hutchinson, J. M. C. (1990). Control of gastropod shell form via apertural growth rates. *Journal of Morphology* **206,** 259-264.

Iijima, A. (2001). Growth of the intertidal snail, *Monodonta labio* (Gastropoda, Prosobranchia) on the Pacific coast of central Japan. *Bulletin of Marine Science* **68,** 27-36.

Janson, K. (1982). Genetic and environmental effects on the growth rate of *Littorina saxatilis*. *Marine Biology* **69,** 73-78.

Janson, K. (1983). Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. *Oecologia* **59,** 58-61.

Johannesson, B. (1986). Shell morphology of *Littorina saxatilis* Olivi: the relative importance of physical factors and predation. *Journal of Experimental Marine Biology and Ecology* **102,** 183-195.

Johannesson, B. & Johannesson, K. (1996). Population differences in behaviour and morphology in the snail *Littorina saxatilis*: phenotypic plasticity or genetic differentiation? *Journal of Zoology* **240,** 475-493.

Johannesson, K., Rolán-Alvarez, E. & Erlandsson, J. (1997). Growth rate differences between upper and lower shore ecotypes of the marine snail *Littorina saxatilis* (Olivi) (Gastropoda). *Biological Journal of the Linnean Society* **61,** 267-279.

Johnson, M. S. & Black, R. (1998). Effects of habitat on growth and shape of contrasting phenotypes of *Bembicium vittatum* Philippi in the Houtman Abrolhos Islands, Western Australia. *Hydrobiologia* **378,** 95-103.

Kemp, P. & Bertness, M. D. (1984). Snail shape and growth rates: evidence for plastic shell allometry in *Littorina littorea*. *Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences* **81,** 811-813.

Kitching, J. A. & Lockwood, J. (1974). Observations on shell form and its ecological significance in thaisid gastropods of the genus *Lepsiella* in New Zealand. *Marine Biology* **28,** 131-144.

Kitching, J. A., Muntz, L. & Ebling, F. J. (1966). The ecology of Lough Ine XV. The ecological significance of shell and body form of *Nucella*. *Journal of Animal Ecology* **35,** 113-126.

Krist, A. C. & Lively, C. M. (1998). Experimental exposure of juvenile snails (*Potamopyrgus antipodarum*) to infection by trematode larvae (*Microphallus sp.)*: infectivity, fecundity compensation and growth. *Oecologia* **116,** 575-582.

Langerhans, R. B. & DeWitt, T. J. (2002). Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. *Evolutionary Ecology Research* **4,** 857-870.

Levins, R. & Lewontin, R. (1980). Dialectics and reductionism in ecology. *Synthese* **43,** 47-78.

Levri, E. P., Dillard, J. & Martin, T. (2005). Trematode infection correlates with shell shape and defence morphology in a freshwater snail. *Parasitology* **130,** 699-708.

McCarthy, H. O., Fitzpatrick, S. M. & Irwin, S. W. B. (2004). Parasite alteration of host shape: a quantitative approach to gigantism helps elucidate evolutionary advantages. *Parasitology* **128,** 7-14.

McNair, C. G., Kier, W. M., LaCroix, P. D. & Linsley, R. M. (1981). The functional significance of aperture form in gastropods. *Lethaia* **14,** 63-70.

Mouritsen, K. N., Gorbushin, A. & Jensen, K. T. (1999). Influence of trematode infections on *in situ* growth rates of *Littorina littorea*. *Journal of the Marine Biological Association of the United Kingdom* **79,** 425-430.

Okamoto, T. (1988). Analysis of heteromorph ammonoids by differential geometry. *Palaeontology* **31,** 35-52.

Oyama, S. (2000). Causal democracy and causal contributions in developmental systems theory. *Philosophy of Science* **67,** S332-S347.

Palmer, A. R. (1990). Effect of crab effluent and scent of damaged conspecifics of feeding growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). *Hydrobiologia* **193,** 155-182.

Palmer, A. R. (1992). Calcification in marine mollusks: how costly is it? *Proceedings of the National Academy of Sciences of the United States of America* **89,** 1379-1382.

Parsons, K. E. (1997). Contrasting patterns of heritable geographic variation in shell morphology and growth potential in the marine gastropod *Bembicium vittatum*: Evidence from field experiments. *Evolution* **51,** 784-796.

Paul, C. R. C. (1991). The functional morphology of gastropod apertures. In *Constructional Morphology and Evolution* (ed. N. Schmidt-Kittler and K. Vogel), pp. 127-140. Springer Verlag, Berlin, Heidelberg.

Picken, G. B. (1980). Distribution, growth, and reproduction of the antarctic limpet *Nacella* (*Patinigera*) *Concinna* (Strebel, 1908). *Journal of Experimental Marine Biology and Ecology* **42,** 71-85.

Raup, D. M. (1966). Geometric analysis of shell coiling: some general problems. *Journal of Paleontology* **40,** 1178-1190.

Rice, S. H. (1998). The bio-geometry of mollusc shells. *Paleobiology* **24,** 133-149.

Rohlf, F. J. & Bookstein, F. L. (2003). Computing the uniform component of shape variation. *Systematic Biology* **52,** 66-69.

Schöne, B. R., Lega, J., Flessa, K. W., Goodwin, D. H. & Dettman, D. L. (2002). Reconstructing daily temperatures from growth rates of the intertidal bivalve mollusk *Chione cortezi* (northern Gulf of California, Mexico). *Palaeogeography Palaeoclimatology Palaeoecology* **184,** 131-146.

Schöne, B. R., Rodland, D. L., Wehrmann, A., Heidel, B., Oschmann, W., Zhang, Z. J., Fiebig, J. & Beck, L. (2007). Combined sclerochronologic and oxygen isotope analysis of gastropod shells (*Gibbula cineraria*, North Sea): life-history traits and utility as a high-resolution environmental archive for Kelp forests. *Marine Biology* **150,** 1237-1252.

Trussell, G. C. (1996). Phenotypic plasticity in an intertidal snail: the role of a common crab predator. *Evolution* **50,** 448-454.

Trussell, G. C. (2000). Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. *Evolutionary Ecology Research* **2,** 803-822.

Trussell, G. C. & Smith, L. D. (2000). Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. *Proceedings of the National Academy of Sciences of the United States of America* **97,** 2123-2127.

Tsoularis, A. & Wallace, J. (2002). Analysis of logistic growth models. *Mathematical Biosciences* **179,** 21-55.

Ubukata, T. (2003). Pattern of growth rate around aperture and shell form in Bivalvia: a theoretical morphological study. *Paleobiology* **29,** 480-491.

Verhulst, P. F. (1838). Notice sur la loi que la population suit dans son accroissement. *Correspondance Mathématique et Physique* **10,** 113-121.

Vermeij, G. J. (1980). Gastropod growth rate, allometry, and adult size: environmental implications. In *Skeletal Growth of Aquatic Organisms: Biological Records of Environmental Change* (ed. D. C. Rhoads and R. A. Lutz), pp. 379-394. Plenum Press, New York.

Vermeij, G. J. (2002). Characters in context: molluscan shells and the forces that mold them. *Paleobiology* **28,** 41-54.

Yeap, K. L., Black, R. & Johnson, M. S. (2001). The complexity of phenotypic plasticity in the intertidal snail *Nodilittorina australis*. *Biological Journal of the Linnean Society* **72,** 63-76.

Zbikowska, E. (2003). The effect of Digenea larvae on calcium content in the shells of *Lymnaea stagnalis* (L.) individuals. *Journal of Parasitology* **89,** 76-79.

Zbikowska, E. & Zbikowski, J. (2005). Differences in shell shape of naturally infected *Lymnaea stagnalis* (L.) individuals as the effect of the activity of digenetic trematode larvae. *Journal of Parasitology* **91,** 1046-1051.

VII. Appendices

Appendix A: Model parameters for figures 1-4.

Parameters \ Fig.	Fig. 1: Ammonite	Fig. 2: Bivalve	Fig. 3: Patella	Fig. 4: Trochita
Aperture shape	Ammonite	Bivalve	Ellipse	Trochita
RoX	10	10	3	4
RoZ	5	10	2	4
T*ix*	-10	-10	0	-10
T*iy*	0	0	0	0
T*iz*	0	0	0	0
Mu	0	-0.5	0	**-55 / -40 / -30**
scale	1.02	1.012	1.02	1.011
thetaX	0	0	0	0
thetaY	0	0	0	0
thetaZ	10	1	**0 / 0.1 / 0.3**	15
Tx in % of *RoX*	-1	-0.05	-0.8	-6
Ty in % of *RoX*	-1	-0.04	-0.5	-1.25
Tz in % of *RoZ*	0	0.1	0	-1.6
Growth rate parameter *r*	**0.01 expo - 0.0189 iso 0.03 expo +**	0.011929 logistic iso	0.023203 logistic+	0.016 expo+
Time at inflexion		**none / 264**	148	
Approximate number of whorls	6	1	0.2	6
Number of iterations	200	440	240	160

In the following **table 2**, the first column of the **anova table** shows the source of the variability (groups). The second shows the Sum of Squares (SS) due to each group. The third shows the degrees of freedom (df) associated with each group. The fourth shows the Mean Squares (MS) for each source, which is the ratio SS/df. The fifth shows the F statistic, which is the ratio of the MS's. The sixth shows the p-value, which is derived from the cdf of F. As F increases, the p-value decreases. A p-value < 0.01 indicates that the slopes or intercepts among groups are not all the same. The **table of parameters' estimation** provides the estimation of slopes and intercepts for each group. For instance, in Table 2a, P1E1 has an intercept equals to 0.01652+0.10416= 0.1207 and a slope equals to 0.90377+(-0.0173) = 0.8865.

Appendix B: Table 2: ***for explanations, see previous page.***

Table 2a: corresponding to figures 10a & 11a

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	0.16393	0.03279	470415.693	0
Shell width (static adult)	1	2.441	2.441	35024511.266	0
Group*Shell width	5	0.001	0.000	1733.8785	0
Error	138	9.62E-6	6.97E-8		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	0.01652	0.0005	32.8699	3.87E-67	
P1E1	0.10416	0.00132	78.9881	9.30E-117	
P1E2	0.10195	0.00084	121.018	5.90E-142	
P2E1	0.01496	0.0009	16.629	9.08E-35	
P2E2	0.00336	0.00127	2.6425	0.0091804	
P3E2	-0.1096	0.00122	-89.672	3.24E-124	
P3E3	-0.1149	0.0011	-104.474	3.06E-133	
Slope	0.90377	0.00017	5355.670	0	
P1E1	-0.0173	0.00049	-34.964	1.91E-70	
P1E2	-0.0167	0.00029	-58.477	2.90E-99	
P2E1	-0.0028	0.00032	-8.7526	6.57E-15	
P2E2	0.00092	0.00041	2.2315	0.027259	
P3E2	0.01702	0.00036	46.9511	9.75E-87	
P3E3	0.0188	0.00036	52.3532	6.42E-93	

Table 2b: corresponding to figures 10b & 11b

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	0.00935	0.00187	7435.3969	0
Shell width (static juvenile)	1	0.81023	0.81023	3220169.306	0
Group*Shell width	5	0.0096	0.00019	760.7621	0
Error	138	3.47E-5	2.52E-7		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	-0.0002	0.00119	-0.1598	0.87329	
P1E1	0.06571	0.00313	20.9617	9.99E-45	
P1E2	0.10052	0.00198	50.726	4.04E-91	
P2E1	-0.00005	0.00216	-0.0241	0.98084	
P2E2	0.0147	0.00299	4.9096	2.54E-6	
P3E2	-0.0654	0.00289	-22.611	3.00E-48	
P3E3	-0.1155	0.00264	-43.684	1.10E-82	
Slope	0.80704	0.00049	1633.326	1.19E-297	
P1E1	-0.0217	0.00142	-15.252	2.12E-31	
P1E2	-0.0376	0.0083	-45.442	6.75E-85	
P2E1	0.00206	0.00093	2.228	0.027498	
P2E2	-0.0045	0.0012	-3.7414	0.00026757	
P3E2	0.02119	0.00108	19.6019	1.04E-41	
P3E3	0.04058	0.00107	38.0241	5.28E-75	

Table 2c: corresponding to figures 10c & 11c

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	0.74513	0.14903	83.5964	0
Shell width (onto cross-sectional)	1	48.4284	48.4284	27165.8713	0
Group*Shell width	5	0.05731	0.01146	6.4299	2.11E-5
Error	138	0.24601	0.00178		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	-0.86176	0.01788	-48.192	3.27E-88	
P1E1	-0.077832	0.03851	-2.0211	0.045202	
P1E2	0.10246	0.04697	2.1813	0.030857	
P2E1	0.0053869	0.04063	0.13257	0.89472	
P2E2	0.029163	0.03681	0.79226	0.42957	
P3E2	-0.011762	0.03958	-0.2972	0.76678	
P3E3	-0.047418	0.03648	-1.2997	0.19586	
Slope	1.1707	0.00727	160.940	6.39E-159	
P1E1	0.084174	0.01726	4.8757	2.94E-6	
P1E2	-0.029334	0.01855	-1.5812	0.11612	
P2E1	0.016464	0.01714	0.96281	0.33833	
P2E2	-0.02584	0.01471	-1.7564	0.08124	
P3E2	-0.042733	0.01444	-2.96	0.0036219	
P3E3	-0.0027304	0.01506	-0.1813	0.85639	

Table 2d: corresponding to figures 10d & 11d

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	2.4454	0.48907	467.6961	0
Shell width (onto longitudinal)	1	175.189	175.189	167531.5847	0
Group*Shell width	5	0.5028	0.10056	96.1641	0
Error	258	0.26979	0.00105		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	-0.99423	0.00748	-132.993	1.09E-239	
P1E1	-0.076377	0.01767	-4.3237	2.19E-5	
P1E2	-0.013143	0.01676	-0.7841	0.43369	
P2E1	-0.054392	0.01758	-3.0935	0.0021957	
P2E2	0.03992	0.01611	2.4774	0.013874	
P3E2	0.083236	0.01542	5.394	1.56E-7	
P3E3	0.020757	0.01664	1.2478	0.21325	
Slope	1.2271	0.0031	395.779	0	
P1E1	0.094346	0.00805	11.7219	1.01E-25	
P1E2	0.035123	0.0072	4.8752	1.90E-6	
P2E1	0.048033	0.00763	6.2927	1.33E-9	
P2E2	-0.036768	0.00634	-5.8015	1.92E-8	
P3E2	-0.098794	0.00555	-17.813	7.88E-47	
P3E3	-0.041941	0.00652	-6.4336	6.02E-10	

Table 2e: corresponding to figures 8a & 11e

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	6.7057	1.3411	9576.756	0
Initial width	1	16.9958	16.9958	121363.9888	0
Group*Initial width	5	0.41004	0.08201	585.6043	0
Error	138	0.01933	0.00014		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	-14.237	0.05148	-276.577	2.8757E-191	
P1E1	4.4687	0.11298	39.5529	3.61E-77	
P1E2	0.31305	0.09899	3.1626	0.0019233	
P2E1	-0.4531	0.11719	-3.8663	0.00016955	
P2E2	1.0568	0.1251	8.4477	3.70E-14	
P3E2	-6.2144	0.12586	-49.378	1.37E-89	
P3E3	0.82895	0.10821	7.6604	2.94E-12	
Slope	4.3109	0.01267	340.301	1.12E-203	
P1E1	-1.0279	0.03035	-33.863	1.00E-68	
P1E2	0.10314	0.02559	4.0311	9.14E-5	
P2E1	0.12363	0.02946	4.1966	4.82E-5	
P2E2	-0.2352	0.03052	-7.7069	2.28E-12	
P3E2	1.31777	0.02823	46.6832	2.05E-86	
P3E3	-0.2814	0.02534	-11.103	7.28E-21	

Table 2f: corresponding to figures 8f & 11f

Anova table	d.f.	Sum Sq	Mean Sq	F	Prob>F
Group	5	96.7134	19.3427	139.9671	0
Initial width (random)	1	211.3171	211.3171	1529.1283	0
Group*Initial width	5	5.2734	1.0547	7.6319	2.33E-6
Error	138	19.0708	0.13819		
Table of parameters' estimation	Estimate	Std. Err.	T	Prob>|T|	
Intercept	0.69055	0.097364	7.0924	6.26E-11	
P1E1	-0.17493	0.252448	-0.69287	0.48956	
P1E2	-0.60692	0.21648	-2.8036	0.0057819	
P2E1	-0.03222	0.2167	-0.14866	0.88204	
P2E2	-0.03912	0.22621	-0.17294	0.86295	
P3E2	0.6577	0.19646	3.3478	0.0010501	
P3E3	0.19549	0.19251	1.0155	0.31166	
Slope	0.64693	0.019436	33.2843	8.34E-68	
P1E1	-0.18895	0.05795	-3.2605	0.0014008	
P1E2	0.03938	0.04207	0.93606	0.35088	
P2E1	-0.11616	0.044134	-2.6313	0.0094725	
P2E2	0.039401	0.042713	0.92247	0.35789	
P3E2	0.17247	0.033303	5.1787	7.74E-7	
P3E3	0.053831	0.036385	1.4795	0.14129	

Chapter 5 - Growth dynamics and shape of molluscan shell: a case study with a population of *Hexaplex trunculus* (Muricidae, Gastropoda) reared in laboratory

Abstract

The comparison of shell shape between and within different clades of molluscs can be informative with regards to the basic rules of accretionary growth. In some ammonoids species, the spacing between growth halts covaries with aperture allometry and the intensity of ornamentation. To test whether this recurrent pattern of variation could reflect basic constraints tied to accretionary growth, we investigate the ontogenetic patterns of covariation among these shells characters in a population of gastropods (*Hexaplex trunculus*, Muricidae) originated from a single egg mass. Growth and shape of individuals are recorded from the age of approximately 100 days to 550 days after hatching, corresponding to the second to fourth/fifth whorl.

Variation in growth rhythm (frequency and amplitude of pulses of growth), in growth rates (e.g. mm shell length per day) and in shape of growth curves (presence/absence of quiescent phase) is extensive. The temporal evolution of various linear measurements is best fitted by third degree polynomials. We differentiate three cases: linear growth curves, growth curves showing off a quiescent phase with or without growth rebound. About 20 percent of the snails in the sample exhibit a quasi-constant growth rate whereas the remaining 80 percent exhibit a more or less marked quiescent phase with reduced or no growth at all. For 70 percent of the snails in the sample, growth resumes after the quiescent phase within the time range of investigation. The time spent on a growth halt seems to increase exponentially with age. Over the duration of the experiment, the mean growth rates are not different among tank replicas or among 'growth curves types' although they can be different on smaller time scales.

Variation in shell shape is analysed by geometric morphometrics of landmarks based on the aperture. We document an ontogenetic allometry of aperture, which becomes relatively wider with size. This is consistent with results obtained using elliptic Fourier analysis of aperture contour or traditional biometrics. Variation in the 'strength of ornamentation' is related to mean spacing between growth halts, spinier snails tending to have more widely spaced growth halts.

The mean number of growth halts per month is related to the global shape of growth curves and to the mean spacing between growth halts: the more frequent the pulses of growth, the shorter the time spent on a growth halt (nearly continuous growth), the more linear the growth curves and the smaller the growth segments between successive growth halts.

This study highlights a covariation among growth rhythm, growth halts spacing, aperture allometry and intensity of ornamentation. In particular, variation in growth rhythm is regarded as critical in generating the observed covariation between growth halts spacing and ornamentation. A growth vector model is used to simulate the formation of growth halts phenomenologically. Regardless of variation in overall growth rates and in growth rhythm during ontogeny, this model

is able to account for the covariations among shell characters observed in this population. Such covariations are proposed to mainly result from simple scaling between the aperture dimensions and the lengths of shell segments between successive growth halts.

Key words: gastropods – growth – allometry – intraspecific variation – geometric morphometrics – growth halts – covariation – Buckman's law of covariation.

List of abbreviations

Growth curves:

- Polynomial:

p_1 / p_2 / p_3 / p_4: estimated parameters of third degree polynomial fitting between 100 and 560 days after hatching (92 snails).

T_{min}: time of minimal growth rate estimated by third degree polynomial fitting (59 snails).

GR_{min}: minimal growth rate estimated by third degree polynomial fitting (59 snails).

- Von Bertalanffy:

b_1 / b_2 / b_3: estimated parameters of Von Bertalanffy fitting between 100 and 400 days after hatching (92 snails).

MeanGRB: mean growth rate estimated by Von Bertalanffy fitting (92 snails).

MS: mean size (shell length) estimated by Von Bertalanffy fitting (92 snails).

- Verhulst:

l_1 / l_2 / l_3: estimated parameters of Verhulst (logistic) fitting between hatching and 400 days after hatching (92 snails).

t_0: mean hatching date (October 7th 2002).

T_{max}: time of maximal growth rate estimated by Verhulst fitting (92 snails).

GR_{max}: maximal growth rate estimated by Verhulst fitting (92 snails).

MeanGRL: mean growth rate estimated by Verhulst fitting (92 snails).

Landmark data:

RW_1 / RW_2 / RW_3 / RW_4: relative warps 1, 2, 3 & 4 respectively (834 apertures, 151 snails).

MRW_1 / MRW_2 / MRW_3: mean relative warp estimated by fitting the scores of each snail on warps 1, 2 & 3 respectively against time between 100 and 400 days after hatching (92 snails).

U_1 / U_2: uniform component 1 & 2, respectively (834 apertures, 151 snails).

MU_1 / MU_2: mean uniform component estimated by fitting the scores of each snail on uniform components 1 & 2 respectively against time between 100 and 400 days after hatching (92 snails).

CS: aperture centroid size (834 apertures, 151 snails).

Outline data:

PF_1 / PF_2 / PF_3 / PF_4: principal factor 1, 2, 3 & 4 respectively (834 apertures, 151 snails).

MPF_1 / MPF_2: mean principal factor estimated by fitting the scores of each snail on principal factors 1 & 2 respectively against time between 100 and 400 days after hatching (92 snails).

Traditional data:

SL: shell length (apex to siphon).

SW: maximal width, perpendicular to shell length.

SR: shell shape ratio: *SL* / *SW*.

Spiral data:

MGHT: mean number of growth halts per month between 100 and 400 days after hatching (92 snails).

MGHS: mean spacing between successive growth halts estimated as the median of angles between successive growth halts built between 150-350 days after hatching (92 snails).

ID: identification number of each snail corresponding to the time of capture.

DAH: number of days after mean hatching date (t_0).

MDAH: average days after hatching.

Contents

I. Introduction

The comparison of shell shape between and within different clades of molluscs can be informative with regards to the basic rules of accretionary growth. In particular, it has been pointed out that common rules of accretionary growth could underlie the morphogenesis of the shell and its evolution in ammonoids and gastropods (Bucher & Guex, 1990; Bucher *et al.*, 1996; Bucher, 1997; Checa & Jimenez-Jimenez, 1997; Checa, Jimenez-Jimenez & Rivas, 1998; Checa, Okamoto & Keupp, 2002). Evidences come from the comparison of intraspecific and/or interspecific patterns of covariation between shell characters (Westermann, 1966; Morita, 1991a, b; Dagys & Weitschat, 1993; Checa *et al.*, 1996; Dagys, Bucher & Weitschat, 1999; Morita, 2003; Hammer & Bucher, 2005a), from the description of changes occurring at maturity in different species or clades (Thompson, 1952; Burnaby, 1966; Bucher, 1997; Chirat *et al.*, 2008) and from the analysis of teratological shells in response to injuries (Guex, 1967; Guex, 1968; Bayer, 1970; Landman & Waage, 1986; Bond & Saunders, 1989; Bucher, 1997; Hammer & Bucher, 2005b) or to change in living conditions (Linsley, 1977; Checa & Jimenez-Jimenez, 1997; Checa, Okamoto & Keupp, 2002).

All this work pointed out that molluscan shell shape variation was remarkably structured, for similar patterns of covariation among shell characters could be found in distinct living or extinct species. Some studies highlighted the generic rules underlying the morphogenesis of the molluscan shell, using either geometrical (Thompson, 1952; Raup, 1966; Okamoto, 1988; Illert, 1990; Savazzi, 1990; Rice, 1998; Hammer & Bucher, 2005b; and see chapters 3 & 4), mechanical (Morita, 1991a, b; Hammer & Bucher, 2005a) or chemical arguments (Hammer & Bucher, 1999; Guex *et al.*, 2003; Hammer & Bucher, 2005b). Some studies laid emphasis on the role of life orientation in the determination of the direction of growth (Linsley, 1977, 1978; Checa & Jimenez-Jimenez, 1997; Checa, Okamoto & Keupp, 2002). Some other studies suggested a role for the preceding whorl in the regulation of coiling (Hutchinson, 1989; Checa, Jimenez-Jimenez & Rivas, 1998; Morita, 2003).

The comparison of patterns of variation of shell shape and its associated growth features, particularly growth halts can highlight some of the rules underlying shell growth. In ammonoids, nautiloids, bivalves and gastropods, many shells exhibit growth halts which are temporary apertures usually exhibiting strong ornaments (spines, tubercles, flares). Some parts of the aperture or spines on the preceding whorl can be partly dissolved with subsequent growth. When growth at the aperture stops (no spiral and no radial growth) the shell can be thickened and some teeth may develop (Spight & Lyons, 1974).

The processes of growth halts formation remain largely a mystery. In the Muricidae *Ceratostoma foliatum*, the number of growth halts per whorl decreases during ontogeny and the temporary apertures progressively acquire new characteristics (reinforcement, closed teeth). Spight & Lyons (1974) documented the growth curves of four snails in this species (shell length against time), illustrating that snails larger than 40 mm could stop growing in shell length for several months (2-4 months) when a temporary aperture has begun to be formed (quiescent phase). Periods of growth halts formation were not detectable in smaller snails. Spight & Lyons (1974) reported that availability of food could affect the duration of these quiescent phases; the poorer the conditions, the longer the non-growth

periods. Illert (1981) suggested that in Muricidae, no growth in shell length was occurring in periods of intense activities (e.g. search for feeding) while periods of resting and immobility (e.g. foraging behaviour) were coinciding with periods of growth.

Bucher (1997) suggested that a regular/irregular spacing between successive growth halts ('constant' angle or not) during ontogeny could be related to isometric/allometric growth of the aperture in ammonoids and gastropods. For instance, at maturity, growth halts approximation is generally associated with changes in aperture shape and/or coiling in ammonoids and gastropods. Among other examples, the shell of gastropod *Epitonium scalare* is isometric (or nearly so) until maturity which is recorded in the shell by a more elliptic aperture and a few (about 5) approximated growth halts. In Muricidae, growth halts can also be more closely spaced at maturity. For instance, in *Bolinus brandaris*, a decrease in the length of spines is coinciding with the last two approximated growth halts.

In highly variable ammonoids species, aperture shape and ornamentation tend to covary with the spacing between growth halts during ontogeny and within species (Bucher, 1997). For instance, in *Gymnotoceras rotelliformis*, growth halts and ribs are more closely spaced in the compressed variants than in the depressed variants (see Introduction chapter). Specimens tend to become more compressed during ontogeny while the number of growth halts per whorl tends to increase. These patterns of covariation among growth halts spacing, ornamentation and aperture shape are quite frequent in the Triassic subfamilies Berichitinidae and Paraceratitinae (Monnet & Bucher, 2005).

Molluscan shell shape has been the focus of extensive theoretical work (e.g. Thompson, 1952; Raup, 1966; Okamoto, 1988; Illert, 1990; Savazzi, 1990; Rice, 1998; Hammer & Bucher, 2005b). However, relatively few is known on the dynamics of shell growth experimentally. Moreover, most theoretical models have not been devised to incorporate these potential experimental results. The goal of this study is twofold. On one hand, we will describe the growth dynamics and shell shape of individuals in a population of gastropods (*Hexaplex trunculus*, Muricidae) originated from a single egg mass. We will also characterize the patterns of covariation among shell characters to test whether they are comparable to the above mentioned patterns of variation in some ammonoid species. On the other hand, we will incorporate some of these new experimental data in a model that has been previously developed to investigate growth dynamics (see chapters 3 & 4).

II. Materials and methods

(1) Specimens

(a) Distribution

Hexaplex (Trunculariopsis) trunculus (Muricidae, Gastropoda), synonymous with *Phyllonotus trunculus* (Linnaeus, 1758) is a temperate marine prosobranch gastropod, distributed in the Mediterranean Sea and Eastern Atlantic Ocean from the Portuguese Coast to the Canary Archipelagos (see Houart, 2001). This species occurs in the inter-tidal and infra-littoral zones up to 100 meters depth. *Bolinus brandaris* (Muricidae, Gastropoda) shows a similar geographical distribution though it is perhaps restricted to less profound depths (up to 50 meters according to Martín, Sánchez & Ramón, 1995). Although the distribution of both species globally overlaps, *H. trunculus* preferentially inhabits rocky

substrates protected by algae whereas *B. brandaris* rather lives buried in sand or muddy substrates[1] (Houart, 2001). Both species are of commercial value, especially in Spain and Portugal. These species are most studied for the sexual abnormality known as imposex (superimposition of male sexual characters onto females, Smith, 1971), which is due to tributyltin used as biocide in anti-fouling paints of boats and ship's hulls (e.g. Ramón & Amor, 2001; Ramón & Amor, 2002; Vasconcelos, Gaspar & Castro, 2006). The alteration of the genital tracts in females can affect population dynamics in these species since sterilization can occur in most advanced stages of imposex development.

(b) Reproduction

H. trunculus and *B. brandaris* are direct developing species, meaning that there is no planktonic larval stage (lecithotrophic development). The metamorphosis happens in the egg capsule. Intracapsular development is supported by nutritive eggs and vitelline material (Bandel, 1975). Recently hatched juveniles are carnivorous. Both species feed on bivalves and other gastropods.

One large egg mass of *H. trunculus* (about 20 cm length) was collected by divers of the laboratoire océanographique Arago (Banuyls-sur-Mer, France) during summer 2002 (late August) along the Western Mediterranean coast at Banyuls-sur-Mer (Réserve naturelle, 40 meter depth). It is likely that this egg mass has been laid down by several females because *H. trunculus* often engages in communal egg laying like other muricacean snails (Muzavor & Morenito, 1999; Romero, Gallardo & Bellolio, 2004; Vasconcelos *et al.*, 2004). Fishermen reported that the reproduction of *H. trunculus* and *B. brandaris* seems to occur twice a year (February/March and July/August). Authors reported that spawns of *B. brandaris* were occurring in April and June/July (Ramón & Amor, 2002) and Martín, Sánchez & Ramón (1995) found egg masses of *B. brandaris* along the Catalan coast in August. Vasconcelos *et al*. (2004) report that, in the Ria Formosa lagoon (southern Portugal), the spawning period generally occurs between February and June. The two adult females of *H. trunculus* housed in laboratory during 6 years also spawn each year in February and July. Once, a female of *B. brandaris* spawn on the same egg mass than that of *H. trunculus* in the laboratory[2] (Fig. $1A_1$).

Like many muricacean snails, the females of *H. trunculus* and *B. brandaris* can store sperm for a long period in the *receptaculum seminis* before fecundation and spawning take place (Fretter & Graham, 1994; Ramón & Amor, 2002). In the lab, a female of *H. trunculus* gave rise to fecund eggs in July 2003, whereas no male has been in contact with it for more than one year. But all the subsequent spawn were not fecund. Also, among 40 snails of *B. brandaris* housed in the same tank, only one spawn has been recorded over 6 years and it was not fecund. It is not known whether the 40 snails of *B. brandaris* housed in the laboratory were all females or whether these snails were affected by imposex.

The capsules of both species are lingulate (tongue-shaped), with vasiform sutures (D'Asaro, 1988; D'Asaro, 1991). The capsular body of *H. trunculus* measures about 7 mm in

1 It is known that *B. brandaris* should be carefully washed before being cooked because of the presence of sand around the foot and in the palleal cavity. The differences in behaviour between both species were also noted under laboratory conditions. Most specimens of *B. brandaris* lived buried in the sandy substrate. A few were rather fixed on the tank walls or tank cover, particularly above the water level if they could have this possibility (alternatively, only the siphon could emerge in the tank corners). *H. trunculus* was rather found fixed to the tank walls, preferentially below the water level, or on rocks.

2 The possibility of mixed-species spawn between *Bolinus cornutus* (Linné, 1758) and *Phyllonotus* species has also been reported in the literature (Knudsen, 1950; D'Asaro, 1991).

length with a breadth of about 3 mm. The surface of *H. trunculus* egg capsule is pale yellowish and its concave side is striated in the direction of its length (D'Asaro, 1991, Fig. 1 A_1, bottom). However, we observed that the intensity of striation was dependent on the female laying the eggs. The egg capsule of *B. brandaris* is more rounded than that of *H. trunculus*, pale pinkish and shows a drop on its concave side (Fig. $1A_1$, top). On the convex side of the egg capsules, a rounded escape aperture closed by a mucoid plug is present. The metamorphosed juveniles leave the capsule by perforating this plug (Fig. $1A_2$).

Vasconcelos *et al.*, 2004 report an important variation in egg size, number of eggs per capsule and number of hatching juveniles per capsule. In the spawn of July 2003, we observed about one hundred of eggs per capsule. It is extremely less than Vasconcelos *et al.* (2004) who counted 600-800 eggs per capsule. In *H. trunculus*, we observed that up to 20 juveniles emerged from a single egg capsule but the majority of egg capsules gave rise to 5-6 juveniles.

(c) Early development

The snails began to hatch in laboratory at the end of September 2002, about one month after the presumptive date of oviposition (Bandel, 1975; Vasconcelos *et al.*, 2004). After having escaped from the egg capsule (Fig. $1A_2$), the juveniles frequently settled inside the egg mass for a couple of days. Hatching lasted about 4 weeks and the first hatched snails were rather located at the periphery of the egg mass. When the egg mass was separated into smaller pieces, the remaining snails hatched almost synchronously within a few days (end of October 2002).

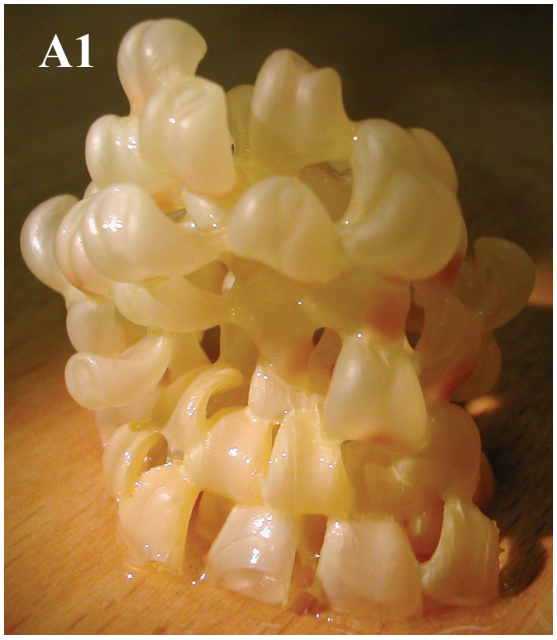
Recently hatched snails were able to float on the water surface by capillarity or even able to swim, perhaps with the help of the remnant of their velum which was sometimes still present a few days after hatching (Fig. 1D). Most snails settled on the tank glasses within a few days after hatching.

Accretionary shell secretion began soon after settlement. The accretionary juvenile shell is distinct from the larval shell. It is characterized by distinct lamellae and spiral striation (Fig. 1C). The transition between the protoconch (larval shell) and the juvenile shell is marked by a thickened lip with a beak situated in a central position on the right side of the aperture (Figs. 1B-C). This larval lip presumably corresponds to the formation of the mantle edge (Kniprath, 1981).

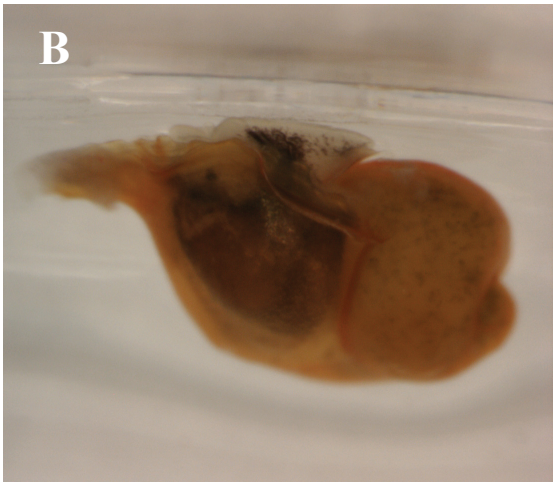
The mantle edge of gastropods is made of two folds of epithelium (Kniprath, 1981; Simkiss & Wilbur, 1989). Along the shell periphery of *H. trunculus*, the mantle edge shows distinct 'lobes' that are in close correspondence with chordal striations and are probably responsible for the genesis of this sculpture. Histological cross sections of the mantle edge of *H. trunculus* along the aperture outline revealed that these 'lobes' are restricted to the outer mantle epithelium (data not shown). As one goes along the aperture, the outer fold is longer than the inner fold at the presumptive place of chordal striations and spines, and of nearly equal length in the presumptive inter-chordal regions.

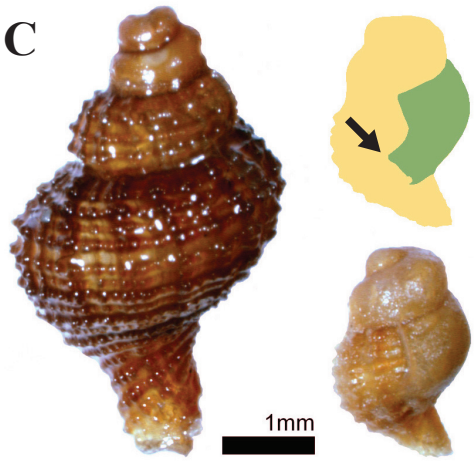
Some variation in the shape of the protoconch is notable. Most snails have 1.5 protoconch

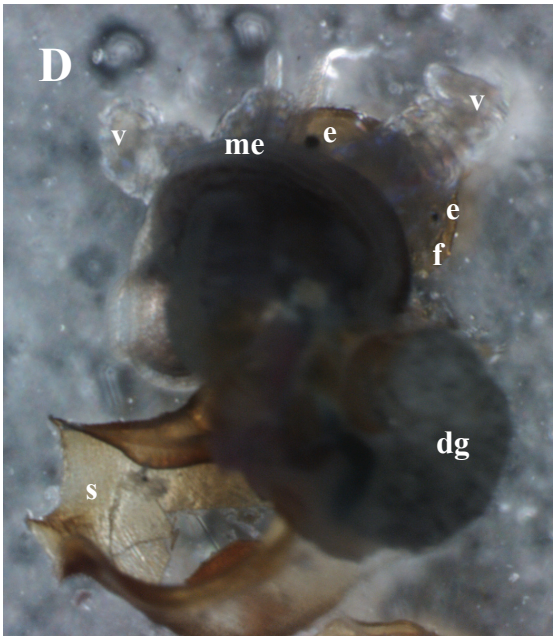
Fig. 1: **A_1**: small egg mass (July 2003) showing the concave side of egg capsules of *H. trunculus* (bottom) and *B. brandaris* (top). **A_2**: convex side of egg capsules of *H. trunculus* showing the escape apertures and hatching juveniles (August 2003). **B**: recently hatched juvenile (fixed to a side of a Petri box) showing the thickened larval constriction and pigmented foot. **C**: differences in size on the 15th October 2002 between snails hatched from the same egg mass. The larval constriction is redrawn. **D**: a recently hatched juvenile with remnant of the lobed velum (note its shell has been unfortunately broken). **dg**: digestive gland; **e**: eyes; **f**: foot; **me**: mantle edge; **s**: siphon; **v**: velum. **E**: drilled shell due to juvenile cannibalism.

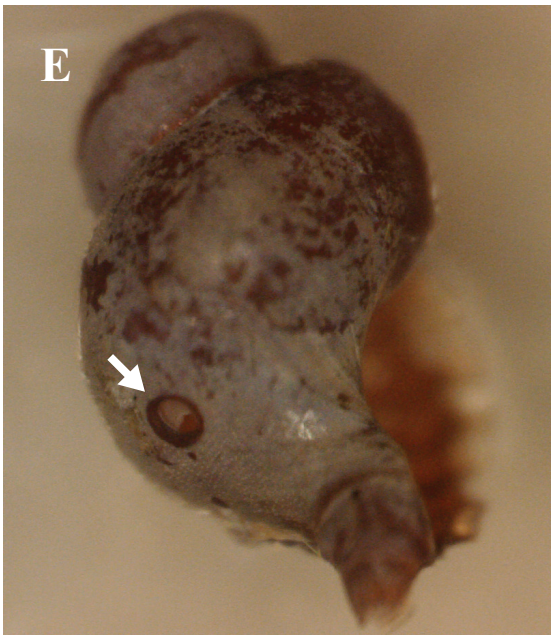

A1


A2


B


C
1mm


D
v
me
e
v
e
f
dg
s


E

whorls[3] but a small proportion of snails can have up to 2.5 whorls. These values are similar to that reported by Vasconcelos *et al.* (2004). The shape of the protoconch whorls is extremely variable: the whorls may be more or less rounded and can exhibit some irregular folds. This can result from the close packing of juveniles into the egg capsules. Also, sometimes, the 'coiling axis' of the protoconch is not aligned with that of the juvenile shell. At hatching, variation in size is not extensive (about 15% of measured value and see below) compared to what we found at later time of ontogeny. Because of hatching asynchrony, on October 15th 2002, some snails had already built two whorls succeeding the larval constriction whereas some others were still into the egg capsules (Fig. 1C).

At hatching, all snails were light to dark brown. But in the second whorl after larval constriction, some snails became light orange (about 30%) whereas the others remained uniformly dark brown. In the third whorl (about 8 months after hatching), shell pigmentation could change again, with (two to four) spiral bands of lighter colour appearing on the previously uniform brown or orange shells. Soon after hatching, the mantle and foot became pigmented (e.g. Fig. 1B).

(d) Experimental design

During the first month after hatching, morta-lity was high (about 50%). This high mortality rate was primary due to predation among the recently hatched juveniles as attested by a high abundance of drilled shells (about 50 % of empty shells, Fig. 1E). Also, the snails seemed to have difficulties in accessing the food sources in the tank and the non-settled snails were easily caught in tank filters. Vasconcelos *et al.* (2004) report a similar cannibal behaviour, indicating that the snails apparently refused all types of food present in the tanks in the first weeks of development.

Once the snails had settled on the tank glasses, they were caught and isolated in numbered plastic containers (cylinders of 50 mm in external diameter, 44 mm in internal diameter, 60 mm in height) closed at both ends by a fine mesh hold by elastic bends. Mortality rate drop down to zero as the surviving snails were isolated in rearing container. The total number of bred snails was 158. These snails were distributed in six tank replicas of 60 litres capacitance each. *Tanks 1, 2, 3, 4* were filled with snails hatched from end of September 2002 to mid October and isolated in plastic boxes during October. *Tanks 5 & 6* were mainly filled with snails hatched at the end of October and isolated in plastic boxes at that time. The density of snails in each tank replica was 27 ± 2 specimens. The identification number of each snail (*ID*) corresponds to the time of capture. Snails with a small number (smaller than 60) are those that spent the longest time free in the hatching tank (up to three weeks against a few days for *tanks 5 & 6*). The medians of *ID* in *tanks 1*, *2*, *3*, *4* & *6* are 66, 45, 33, 65 & 144 respectively.

The snails were bred in controlled purified aerated sea water conditions (1200 g/L salinity, 21°C, pH 9.3-9.6) and abundantly fed weekly and individually on frozen shrimps pieces (black tiger)[4]. The position of the snails in the tanks was randomly changed at the time of feeding. The temperature of the room was thermostatically regulated at 19 ± 1°C (water temperature of 20 ± 1°C) and lights provided a natural photoperiod.

3 The number of whorls was deduced from the spiral length of the embryonic shell (see Fig. 2D). The beginning of the spiral curve was approximated by a circle. Subsequent rotation was counted and transformed into number of whorls.

4 ≈ 3/4 to 3/2 shrimps per tank according to snail sizes. Snails were fed *ad libitum*.

On a monthly basis, the water was controlled for its pH, its concentration of nitrates (NO_3^-, NO_2^-), calcium (Ca^{2+}) and phosphate (PO_4^-). If the level of nitrates was found within a reasonable range (NO_3^-< 25 mg/l and NO_2^-<0.8 mg/l), 25-33 % of water was changed. If not, 25-33 % of water was changed again two weeks later to return to earlier levels. Each freshly prepared water bucket was equally distributed among the tank replicas. Every two weeks, the tanks were cleaned for algae, which developed rapidly in spring-summer. No difference in the water quality across tanks was found (salinity, nitrates, calcium and phosphate), although tanks developed variable amount of algae, sometimes of different types (red or green algae).

(2) Data acquisition: Automated detection of aperture outlines and landmarks

(a) Data set

Pictures of the living shells in apertural and apical views were taken on a monthly basis using a digital CCD camera (Nikon DXM1200) mounted on a Nikon SMZ1500 stereomicroscope coupled with software ACT1. For this operation, the specimens were gently dried and immobilized with typing machine adhesive. All snails were photographed 10 to 12 times during the study period (about 100 to 550 days after hatching, hereafter *DAH*), except for the snails in *tank 5* which were photographed only twice to control for the side-effects of manipulation on growth and shape. The aperture of the snails was marked with a coloured nail polish in order to record its position at about 150 *DAH*.

Seven snails (5 % of the dataset) became teratological after manipulation (broken siphon, or broken shell edge). They were removed from the study. A preliminary analysis revealed that aperture shape could be more different between two growth halts than on subsequent growth halts, especially for the snails exhibiting widely spaced growth halts[5]. Then, the data on snails which were building a shell segment at the time of photography were excluded from the study. Otherwise, intra/inter-segment variation would blur the overall shape variation pattern.

A technical difficulty was to determine if the snails growing nearly continuously by building many small shell segments were actually in an active phase of growth or not. But for these snails, the change in aperture shape between two growth halts was small (data not shown). It was observed that the proportion of snails actually growing at the time of photography was decreasing if the snails were photographed a 5-6 days after having been fed. So, the snails were fed on Fridays and photographed on Wednesdays and Thursdays. The final sample consists of 834 apertural/apical views pairs of photographs corresponding to 151 snails (7 measurements per snail in average). It represents about 60 % of the initial dataset.

(b) Automated detection of aperture outlines and landmarks

We tried to perform automated segmentation[6] of original shell images in apertural view using basic routines written in Matlab (version 7.1, R14). They provided good results for young snails which where rather uniform in colour and

5 The percentage of variance explained for relative warps in manually digitized data on the whole data set, except teratological snails (1312 apertures) is: $\boldsymbol{RW_1}$: 41 %. $\boldsymbol{RW_2}$: 24%. $\boldsymbol{RW_3}$: 23%. $\boldsymbol{RW_4}$: 11 %. RW_1, RW_2 & RW_3 are confounded with intra/inter-segment variation since the scores of the apertures taken between two growth halts or between successive growth halts are significantly different (RW_1: F = 22.6, p = 2.1587e-006. RW_2: F = 74.8, p = 0. RW_3: F = 94, p = 0. RW_4, F = 1.7, p = 0.18).

6 Segmentation is an image processing operation that is used to select objects in an image. Objects of interest can be detected by algorithms analysing for instance colour or intensity changes of pixels..

texture. However, as the snails grew, the distinct lighter colour bands and wider spacing in growth lamellae (and moderate shell abrasion) greatly affected the performance of these algorithms. At the end, we decided to segment the shells images 'manually' in Photoshop (version 7.0). On the resulting binary images[7], the major axis of the ellipse enclosing the shell region (roughly equivalent to the first harmonic of the elliptic Fourier decomposition) is used to align the shells (orientation normalization; major axis adjusted to the *y*-axis).

Shell length and width were directly obtained on the binary images as the dimensions of the bounding box of the shell region after reorientation (Fig. 2A).

From the binary images, the outlines could be easily retrieved in Matlab by using 8-connected neighbourhood boundary tracing functions, starting from the shell apex (phase normalization) and running in clockwise direction. We computed the distance between each pixel of the shell outline and the centroid of the shell region. The corresponding curve was used to detect the boundaries of the aperture (Fig. 2C, later analysed by Elliptic Fourier, Fig. 2B, see below) as well as the five landmarks used to perform the geometric morphometrics analysis (Fig. 2A).

Landmark 1 is located at the junction between the current aperture and the previous whorl (umbilical suture). *Landmark 2* is situated at the top of the most developed spiral chord, which usually gives rise to a small spine at mid to late stages of ontogeny (3rd to 5th whorls). *Landmark 3* is placed where the convexity of the aperture outline reverses. It represents the junction between the posterior part of the aperture and the siphon. *Landmark 4* is situated at the base of the siphon on the right side of the aperture. *Landmark 5* is placed on the left side of the shell, at the convexity reversal of the left part of the shell outline on the previous whorl. It is also the point of the shell outline which is the closest to the aperture centroid.

To control for the quality of the automated detection of landmarks, we also performed a study using manually digitized landmarks. *Landmarks 1*, *2*, *3* and *4* were located at the same place, but *landmark 5* was placed on the left side of the aperture where the convexity reverses (near the attachment of the columellar muscle) rather than on the previous whorl. The results were qualitatively equivalent to those obtained using the automated digitized landmarks, except that the first relative warp accounted for a smaller percentage of variation[8]. We also noted that *landmarks 3 & 5* had a relatively lower contribution to the variation in the data set in the automatically detected landmarks' case than in the manually digitized landmarks' one[9]. This is expected because landmarks placed at change of convexity in outlines are difficult to define manually, especially when the change of convexity is low. Then, it seems that the automated detection of landmarks has reduced noise associated with the loosely defined *landmarks 3 & 5* (resulting in increasing the relative contribution of other landmarks, especially *landmark 2*). No consistent difference in the interpretation of shape changes along relative warp axes was noted with respect

7 A binary image is defined as a series of 0's and 1's (logical). Pixels with the value 0 are displayed as black; pixels with the value 1 are displayed as white.

8 Percentage of variance explained for relative warps in manually digitized data (834 apertures): $\boldsymbol{RW_1}$: **41 %**. $\boldsymbol{RW_2}$: 28%. $\boldsymbol{RW_3}$: 19%. $\boldsymbol{RW_4}$: 12 %.
Percentage of variance explained for relative warps in automatically digitized data (834 apertures): $\boldsymbol{RW_1}$**: 49 %**. $\boldsymbol{RW_2}$: 23%. $\boldsymbol{RW_3}$: 18%. $\boldsymbol{RW_4}$: 10 %.

9 Relative contribution of each landmark in manually digitized data: $\boldsymbol{LM_1}$: 38%; $\boldsymbol{LM_2}$: 20 %; $\boldsymbol{LM_3}$**: 19 %**; $\boldsymbol{LM_4}$: 5%; $\boldsymbol{LM_5}$**: 18%.**
Relative contribution of each landmark in automatically digitized data: $\boldsymbol{LM_1}$: 40 %; $\boldsymbol{LM_2}$: 35%; $\boldsymbol{LM_3}$**: 14%**; $\boldsymbol{LM_4}$: 5%; $\boldsymbol{LM_5}$**: 7%**.

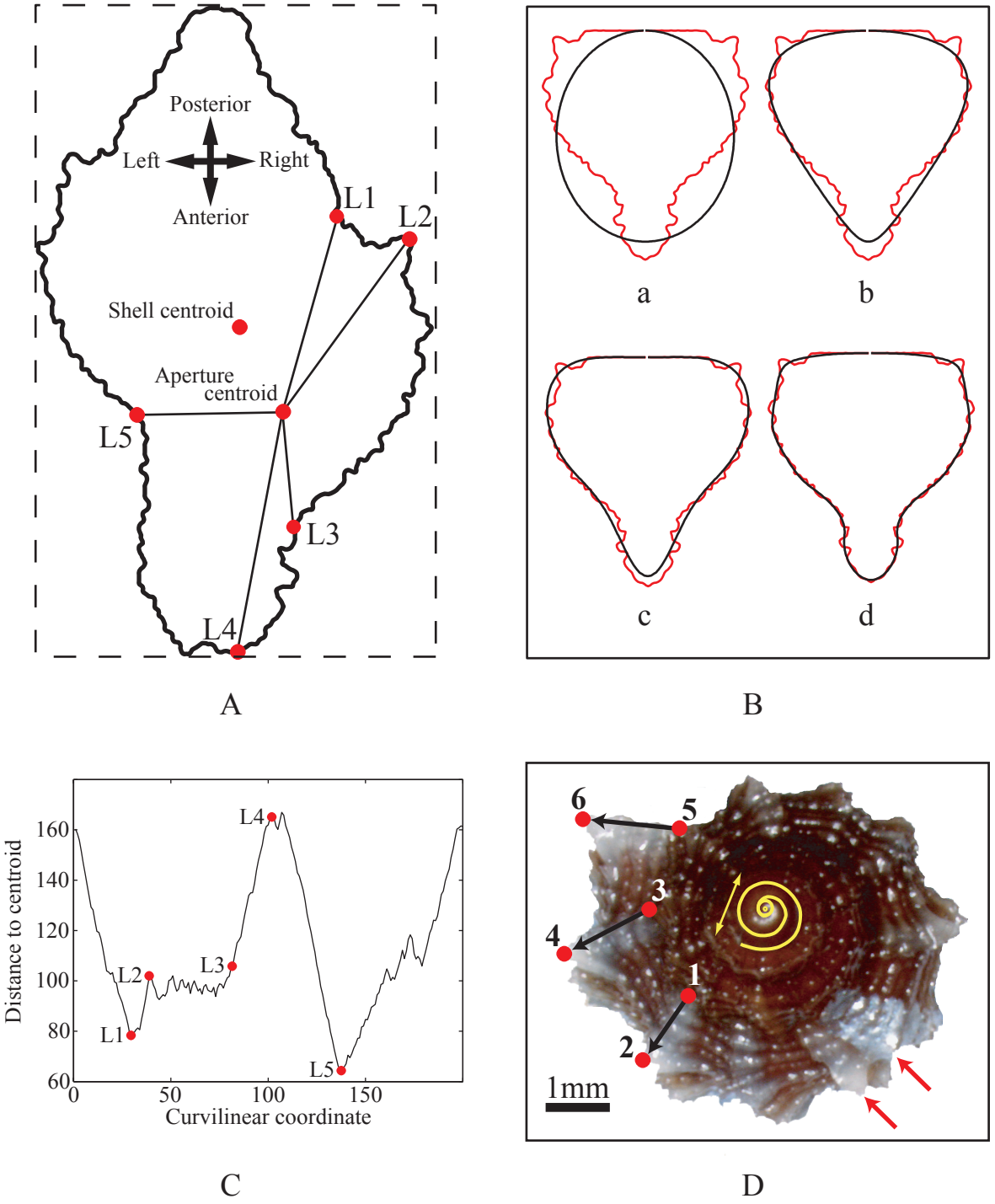


Fig. 2: **A**: shell contour (black) and bounding box which encloses it (dashed line) in apertural view. Shell length (*SL*) and shell width (*SW*) correspond to the length and width of the bounding box, respectively. Shell centroid is the centroid of the shell contour. L_1, L_2, L_3, L_4 & L_5: landmarks used to characterize variation in aperture shape. Aperture centroid is the centroid of these 5 landmarks. Aperture centroid size is the sum of the distances between each landmark and the aperture centroid (thin-black lines).**B**: superimposition of the duplicated aperture outline (red) and its reconstructions (black) with **a**: 1; **b**: 2; **c**: 4 and **d**: 10 elliptic Fourier harmonics. **C**: shape curve used to automatically locate the 5 landmarks (**A**) and the aperture contour (**B,** between L_1 and L_4). This shape curve is the plot of the distances between the pixels of the shell contour and the shell centroid *vs.* curvilinear coordinate (pixel number in clockwise direction starting from shell apex). **D**: calculation of the angles between successive growth halts in apical view. Localization of 'odd' and 'even' landmarks and associated vectors (black arrows) used to calculate the angles between growth segments. The red arrows indicate a probable example of manipulation-induced growth halts, since a growth halt is built just next to the nail polish mark, corresponding to the previous photograph.

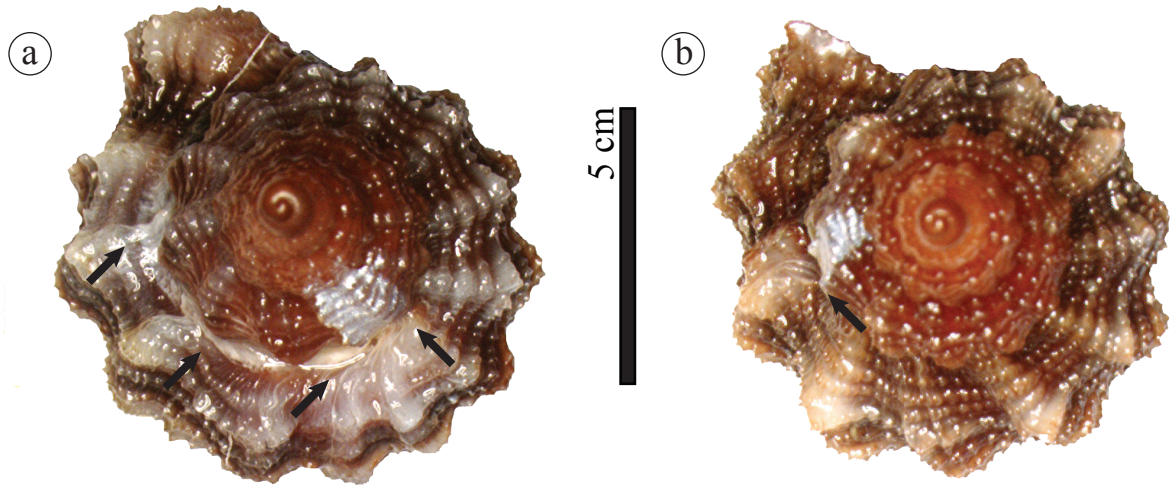


Fig. 3: Examples of anomalous shell growth in response to a nail polish mark on the previous whorl. **a**: in the five shell segments consecutive to the nail polish mark, the whorl is 'detached'. During growth, this small empty space is filled with shell material. The last shell segment is 'normal'. **b**: only one shell segment is affected by the nail polish mark leading to recurved lamellae.

to the placement of *landmark 5* on the previous whorl or on the left side of the aperture. So the coordinates of the automated detected landmarks constitute the morphological dataset discussed in this study (834 apertures corresponding to 151 snails).

On each picture in apical view, landmarks were manually placed on the successive growth halts built after the nail polish mark (Fig. 2D). The 'odd' (numbered) landmarks were placed on the umbilical line whereas the 'even' (numbered) landmarks were placed on the farthest point on the free side of the aperture. The number of growth halts built between successive pictures was counted as the difference in the number of 'even' landmarks minus 1. This number was normalized over one month. The angle between two successive growth halts was computed as the arccosinus of the scalar product of the unit vectors, defined by pairs of successive 'odd-even' landmarks (Fig. 2D). To constrain the size at hatching in some growth curve fittings (see below), we also measured the width of embryonic shell and its spiral length (Fig. 2D). Values for embryonic shell length were estimated from the regression of length and width measurements of dead embryonic shells from the same sample.

(c) Manipulation artefacts and sources of error

A comparison with the snails in the control *tank 5* reveals that manipulation could force some snails which were growing at the time of photography to build a growth halt (e.g. Fig. 2E, arrows). This is particularly obvious for the snails tending to exhibit a rather large spacing between growth halts. For these snails, the spacing between growth halts is rather irregular compared to the specimens in the control tank. Of course, these manipulation-induced growth halts will lead to underestimate the spacing between growth halts (see below).

Another bias is introduced by the orientation of the shell in apical views. Actually, the apical views are not strictly apical. The siphon was pushed into the typing machine adhesive but not too deep in order to prevent shell breakage. As the snails were trying to move, this resulted in balancing the shell according to the weight of the soft parts (toward the antero-right side of the

aperture). The comparison of angles calculated for the same shell segment on successive pictures showed up to 20 percent of error depending on the position of the measured segment relative to the current aperture. As the maximal range of variation in growth halts spacing is 22-42° (corresponding approximately to 16 and 8 growth halts per whorl respectively), and given that the spacing between growth halts tends to increase over time, the uncertainty on the measured angle was nearly as high as the expected variation. For instance, sometimes, growth halts were widely spaced, but because of the error of orientation in apical view, the two successive apertures were nearly parallel, and consequently a small angle between the two successive apertures was calculated. In this study, it was not investigated whether the degree of tangency of the aperture relative to the coiling axis was variable during ontogeny and among snails. Neither was the non-planar character of the apertures taken into account. But if so, it might affect the results for growth halts spacing. For these reasons, it was not possible to compare the temporal evolution of the growth halts spacing among snails. Alternatively, the median of the measured angles (on the picture comprising the largest number of growth segments since the nail polish mark) was taken as an estimate of the mean spacing between growth halts for each snail. It corresponds to the averaging over one whorl growth (3rd to 4th whorl plus/minus half a whorl) as built between 150-350 *DAH* approximately (note that after a full 360° whorl growth, the nail polish was frequently lost). If the nail polish was not lost before a full 360° whorl growth, the whorl could be slightly detached from the previous whorl, giving rise to recurved growth segments (Fig. 3, arrows).

To each point of the growth curves (size *vs.* time) should be assigned a small size uncertainty as well as a more critical time uncertainty or time interval. It comes from the fact that shell growth is discontinuous. For those snails that exhibit the smallest number of growth halts per whorl, the time uncertainty is probably higher than for others. It was observed that for snails of similar shell length, at about 200 *DAH*, the duration of the pause at a temporary aperture was about 15 days for the snails exhibiting the lowest number of growth halts per whorl, against 3-5 days for those exhibiting the largest number of growth halts per whorl. As the snails were measured at the same time within each tank, it cannot be ascertained whether the size measurements were taken roughly midway during the resting phases, especially if these lasted about 15 days.

(3) Methods

(a) Fittings

We fitted several variables against time by non-linear equations, for the snails whose number of measurements was equal or superior to 6 (92 snails). The temporal evolution of aperture centroid size[10] (*CS*), shell length (*SL*) and shell width (*SW*) were fitted by polynomial equations over the whole duration of the experiment[11]. These variables were also fitted by Von Bertalanffy and Verhulst (logistic) equations over a restricted time range (see below). The number of growth halts per month (*GHT*) against time was fitted by

10 The centroid size is the square root of the sum of squared distances of a set of landmarks to their common centroid (Bookstein, 1991). It is proportional to the area covered by a configuration of landmarks, and thus provides a measure of overall size that is uncorrelated with the configuration of landmarks in the presence of isometry.

11 The temporal evolution of *CS* & *SL* was similar. In this study, we preferred to illustrate the growth curves using *SL* as the measure of size, for *CS* is dimensionless and does not easily allow the comparison of growth rates with other studies. Fittings for *CS* and *SW* are displayed in appendices.

a linear regression[12]. Several constraints, based on biologically meaningful hypotheses, were provided to allow the non-linear fits of growth curves to converge (see below). The goodness of the fits was estimated by the coefficients of determination which were superior to 95% in all cases.

Mean size (*MS*) was estimated as the area below the Von Bertalanffy growth curves of shell length (*SL*) over 100-400 *DAH*. The growth rate curves were obtained by derivation of the fitted growth curves. These curves were concordant with the instantaneous empirical growth rates calculated directly from the data. The mean growth rates (*MeanGRB*, *MeanGRL*) were estimated as the area below the growth rate curves derived from Von Bertalanffy and logistic fittings respectively over the duration of interest. Similarly, the mean number of growth halts per month (*MGHT*) was estimated as the area below the regression line of the number of growth halts per month (*GHT*) against time between 100 and 400 *DAH*.

A fitting of the log-transformed instantaneous shell length (*SL*) against instantaneous shell width (*SW*) was performed to obtain the traditional allometric coefficients for each snail. The temporal evolution of derived shape variables (see below) was also fitted by linear regressions for each snail (RW_1 *vs.* time, etc). The area below this regression line provided an estimate of the mean shape for each snail over 100-400 *DAH* along each axis of variation (MRW_1, etc).

12 A preliminary study using particularly long sequences of growth halts (two to three whorls) from 150-550 *DAH* points out that the curve of *GHT versus* time would be better fitted by a slowly decreasing exponential (or a second degree polynomial for snails resuming growth after a quiescent phase, see below) rather than by a straight line. However, in order to keep as many snails as possible in this study (92), including those that lost their nail polish mark after one whorl, we chose to use a linear regression over a restricted time period (150-400 *DAH*). This regression would provide qualitatively similar results for all snails, regardless of the length of the sequence of growth halts.

(b) Geometric morphometrics

One powerful way to study shape variation is to use landmark-based geometric morphometrics (Bookstein, 1991, 1996) which allow not only complete separation of size and shape into distinct variables but also segregation of shape into uniform and non-uniform components as well as practical means to visualize shape changes using thin plate spline (tps) techniques. Moreover, the resulting shape variables can be incorporated into multivariate analyses such as principal components analysis (see below).

Geometric morphometrics describe shape variation as the distribution of specimens (represented by their configuration of landmarks) around a common, distance-minimized, reference form (consensus). Let assume that the data set is made of the two- dimensional (x, y) coordinates of p landmarks (geometrically homologous points) in n specimens. The n configurations of landmarks are superimposed, rotated and scaled using the generalized orthogonal least squares Procrustes analysis (Rohlf & Slice, 1990). The consensus ($p \times 2$) then represents the mean configuration of the p landmarks once the differences due to location, rotation and scaling are removed.

The displacement of each configuration of landmarks with respect to the consensus could be described by many transforms. The one which is usually preferred is the one that minimizes the so-called bending energy, by analogy with the deformation of a thin plate of steel. The sharper the bending of the plate the greater the energy required. A bending energy matrix can be defined from the distances between each pairs of landmarks in the consensus ($p \times p$ matrix). Its principle is that the smaller the distance between any two landmarks in the consensus configuration, the larger the energy required for separating

them. In other words, local deformations require higher energies. Thin-plate splines are used to describe and visualize the transformation of the *n* configurations of landmarks from the consensus.

The behaviour of these splines at infinity corresponds to their affine transformation. The uniform components ($n \times 2$) represent the large scale deformations (along the *x* and *y* axes respectively) that conserve the parallelism between originally parallel lines (Bookstein, 1989, 1991; Rohlf & Bookstein, 2003).

The principal warps ($p \times p$) are the eigenvectors of the bending energy matrix and represent the non-affine transformation of the thin-plate splines (Bookstein, 1989). They are also termed the non-uniform component of shape description because they summarize localized shape variation at variable geometric scales. They are ordered according to smaller and smaller geometric scales; that is to say according to the inverse of their eigenvalue (which corresponds to the bending energy required to account for the deformation of the configuration of landmarks).

The partial warps scores ($n \times 2 \times p$) are obtained by weighting the principal warps by the distances between each configuration of landmarks and the consensus in *x* and *y* directions (Rohlf, 1998).

The relative warps are the linear combination of partial warps that account for the maximum of variation in the sample. The relative warps scores ($n \times 2 \times p$) are obtained by performing a principal component analysis (PCA) on partial warps scores. The relative warps are thus statistically orthogonal to each other, meaning that they represent statistically independent axes of shape variation. It is common to analyse only the first three relative warps which generally account for two third of the variation in the sample. Thin-plate splines are used to visualize shape changes as deformations along each significant relative warp (hereafter RW_1, RW_2, RW_3 & RW_4).

In this study, the relative warps analysis was performed in the tpsRelw program (Version 1.31, 2003). The exponential weighting parameter (alpha) was assigned a value of zero, thus giving equal weight to distances at all scales. The alignment scaling method was made according to unit centroid size and the alignment projection method was set to orthogonal. The uniform component was computed separately from the relative warps analysis.

Note also that in geometric morphometric studies, the already mentioned centroid size, (the square root of the sum of squared distances of a set of landmarks to their common centroid), is traditionally used as an estimate of overall size. This scaling variable is uncorrelated with shape measures in the presence of isometry (Bookstein, 1991) and therefore allows the separation between geometric size and shape.

(c) Elliptic Fourier analysis

Elliptic Fourier analysis (Kuhl & Giardina, 1982) is used to reduce the data of the digitized aperture outlines to a much smaller set of meaningful shape parameters, which, as in geometric morphometrics, can be incorporated into a multivariate analysis. It decomposes a closed contour into a sum of harmonically related ellipses (Ptolemaic epicycles, Ferson, Rohlf & Koehn, 1985; for a geometric representation see also Schmittbuhl *et al.*, 2003 and Fig. 2B).

The Cartesian coordinates (x, y) of a large number of pixels around each outline is required (in this study 200 per aperture outline). The *x* and *y* coordinates are processed separately as parametric functions of the curvilinear coordinate (t)

(position on the outline). Since these two functions are single-valued, continuous and periodic (closed curve), they can be expanded into Fourier series. Each harmonic is coded by four Fourier coefficients, namely the sine and cosine amplitudes of the *x* and *y* increments.

The different harmonics correspond to different spatial scales. Low order harmonics usually code for the 'global' shape, whereas higher order harmonics merely correspond to details of the outline. The more harmonics are used, the more closely the reconstructed outline converges onto the original outline. In this study, the 10 first harmonics were used (Fig. 2B). A principal component analysis (PCA) was performed on the corresponding 40 Fourier coefficients.

The closer the outline shape resembles a smooth ellipse, the lower the number of harmonics required to approximate the initial outline adequately. On the contrary, outlines containing right angles are relatively more 'harmonic consuming'. In that respect, and since the left part of the aperture outline could not be properly digitized and needed to be replaced by some kind of closing, we opted for a solution where the right part of the aperture outline is mirrored about the *y*-axis (Fig. 2B).

(d) Statistical tests

One way analysis of variance (Anova) or its non-parametric equivalent (Kruskal-Wallis analysis variance) were performed on fitted parameters or derived shape variables to test for mean or median differences among groups. We tested for differences among the tank replica. The results are displayed as box-whisker graphs, which illustrate whether the mean or median in each group are regarded as drawn from the same population (if the notches overlap), that is whether or not significant differences among groups can be suggested (5% of significance level).

To identify the statistical correlation between variables, Pearson's *r* was used or alternatively Spearman's *rho* whenever the test for normality of variables failed (Lilliefors' test). Tests were applied on various variables to account for the degree of covariance among them (e.g. shape *versus* size) and to test for the concordance of different fitting equations and methods of shape analysis. Results are presented in the following tables and figures. A PCA was also performed to test for the covariation between growth and shape variables.

III. Results

The first measurements were made on January 15th 2003. The first snails to hatch in end September 2002 cannot have been older than 117 days on January 15th 2003, whereas the last snails to hatch in end of October 2002 cannot have been younger than 77 days at that time. Since the date of hatching is not known for any snail, we chose to place the beginning time of measurement at 100 days after hatching (*DAH*), as if all snails hatched on October 7th 2002 (t_0). This choice leads to overestimating the real duration between hatching-first measurements for snails of *tanks 5 & 6* which hatched almost synchronously at the end of October. Reciprocally, this choice leads to underestimating this duration for the first hatched snails, more or less randomly distributed in other tanks (see below). The snail identification numbers (*ID*) correspond to the time of capture and provide a constraint upon the presumptive date of hatching for each snail (e.g. a snail with an *ID* inferior to 120 could not have hatched later than mid October).

(1) Growth curves

(a) Polynomials

Over the whole range of measurements (100 to 560 *DAH* / 15th January 2003 - 31st March 2004), the temporal evolution of aperture centroid size (*CS*), shell length (*SL*) or shell width (*SW*) has been approximated by a third degree polynomial equation for 92 specimens that had more than 6 data measurements (*tanks 1, 2, 3, 4 & 6*):

$$Y = p_1 X^3 + p_2 X^2 + p3\, X + p_4$$

where Y is a measure of size and X is the number of days between 100 and 560 *DAH* (see Fig. 4a for an example of variation for three snails bred in the same tank).

The derivative of this equation is a second degree polynomial, meaning that the growth rate curve is parabolic. If p_1 is positive, the growth rate first decreases and then increases (Fig. 4d). Without constraints on the polynomial coefficients, about 95 percent of the snails in the sample are relatively well fitted by a positive p_1. It means that at the beginning of measurements (approximately 100 *DAH*), the vast majority of snails were experiencing a decrease in their growth rate.

A second fitting was performed by forcing p_1 to be positive. For all snails, p_2 was negative whereas p_3 and p_4 were positive. The flatness of the growth curve is mainly accounted for by the parameter p_1: the smaller p_1, the flatter the growth curve and the more constant the growth rate.

The time of minimal growth rate (T_{min}) is the time at which the second derivative of the growth curve is null (Fig. 4g):

$$T_{min} = -p_2 / (3 \times p_1);$$

The minimal growth rate (GR_{min}) at that time is:

$$GR_{min} = - p_2^2 / (3 \times p_1) + p_3;$$

The fact that GR_{min} cannot be inferior to zero imposes constraints on the parameters p_1, p_2 and p_3.

If the fitting resulted in finding a negative GR_{min} over the time range of rearing, the parameter p_1 was constrained to be null, thus resulting in a second degree polynomial fitting. The snails well fitted by this second degree polynomial equation (about 10 percent) are those that exhibit only a decrease in growth rate over the time range of rearing. They are the snails that actually did not increase their growth rate before 560 *DAH* and those for which the last measurements were removed from the study (see above). In both cases, T_{min} and GR_{min} cannot be meaningfully estimated since these parameters fall outside the range of data.

Similarly, if T_{min} is negative or superior to 560, it means that no meaningful GR_{min} can be found over the time range of rearing. In these cases, the growth curves are better (or at least equally well) fitted by a linear equation, that is to say that p_1 and p_2 are constrained to be null. The growth rate is assumed to be constant over time and it equals p_3. About 20 percent of the snails in the dataset exhibit a quasi-linear growth curve and hence a rather constant growth rate.

Lastly, parameter p_4 represent the size at 100 *DAH*. The fitting results were improved by constraining this parameter between 2 and 15 mm for *SL*; 1.5 and 10 for *SW*; and 2 and 13 for *CS*, which were the observable range of size variation at 100 *DAH*.

To sum up, about 20 percent of the snails in the sample exhibit a quasi-constant growth rate (poly1) whereas the remaining 80 percent exhibit a more or less easily identified quiescent phase where growth rate unambiguously tends

toward zero (poly2, poly3). For 70 percent of the snails in the dataset, growth rate increases after the quiescent phase in the time range of rearing (poly3). For these latter snails, T_{min} is evaluated between 285 and 350 *DAH* (median for each tank, corresponding to mid-July to mid-September 2003). The medians of T_{min} are not significantly different among tank replicas (Chi-sq = 8.67, p = 0.07, see Table 1 in appendices), although the snails of *tanks 1 & 4* tend to have a delayed T_{min} compared to snails of *tanks 2, 3 & 6* (Fig. 5a). GR_{min} is different among tank replicas (Chi-sq = 14.54, p = 0.006, Table 1). Snails of *tanks 2 & 3* tend to have a higher GR_{min} (median 0.026 mm *SL* / day or 0.78 mm *SL* / month) than those of *tank 4* (median 0.014 mm *SL* / day or 0.42 mm *SL* / month) (Fig. 5c). The GR_{min} of snails in *tanks 1 & 6* are intermediate and not significantly different from the other tanks (median 0.018 mm *SL* / day and 0.022 mm *SL* / day respectively, corresponding to 0.54 mm *SL* / month and 0.66 mm *SL* / month). It means that in *tank 4* (and *tank 1*), the snails tend to have a rather well marked quiescent phase during which there is no growth (GR_{min} can be null) and which can last quite long (until 100 days for some snails).

Indeed, it seems that this variation in growth curves is continuous. Most snails (80 percent) exhibit a relatively well marked quiescent phase. For the remaining 20 percent there are three possibilities: (1)- they do actually exhibit a quiescent phase but it is too short for our monthly measurements to catch it; error of measurements (although small) have the consequence that the better fit is a linear one; (2)- they could exhibit a quiescent phase of similar duration (1-3 months) but not during the time range of investigation (e.g. earlier/later); (3)- they would never exhibit a quiescent phase whatever the time delay between successive measurements and the duration of the experiment. Unfortunately, it is impossible to assert any of these hypotheses but the first and second ones seem the most likely (see below for a discussion).

Comparing the evolution of growth rate with polynomial coefficients is difficult, for meaningful features of growth (T_{min}, GR_{min}) are dependent on several parameters. Moreover, for 30 percent of the snails, these features cannot be evaluated at all. In consequence, growth curves have been 'forced' to follow a common pattern over a restricted time range. They have been respectively fitted by a Von Bertalanffy equation from 100 to 400 *DAH* and by a Verhulst (logistic) equation from t_0 to 400 *DAH*.

(b) Von Bertalanffy's equation

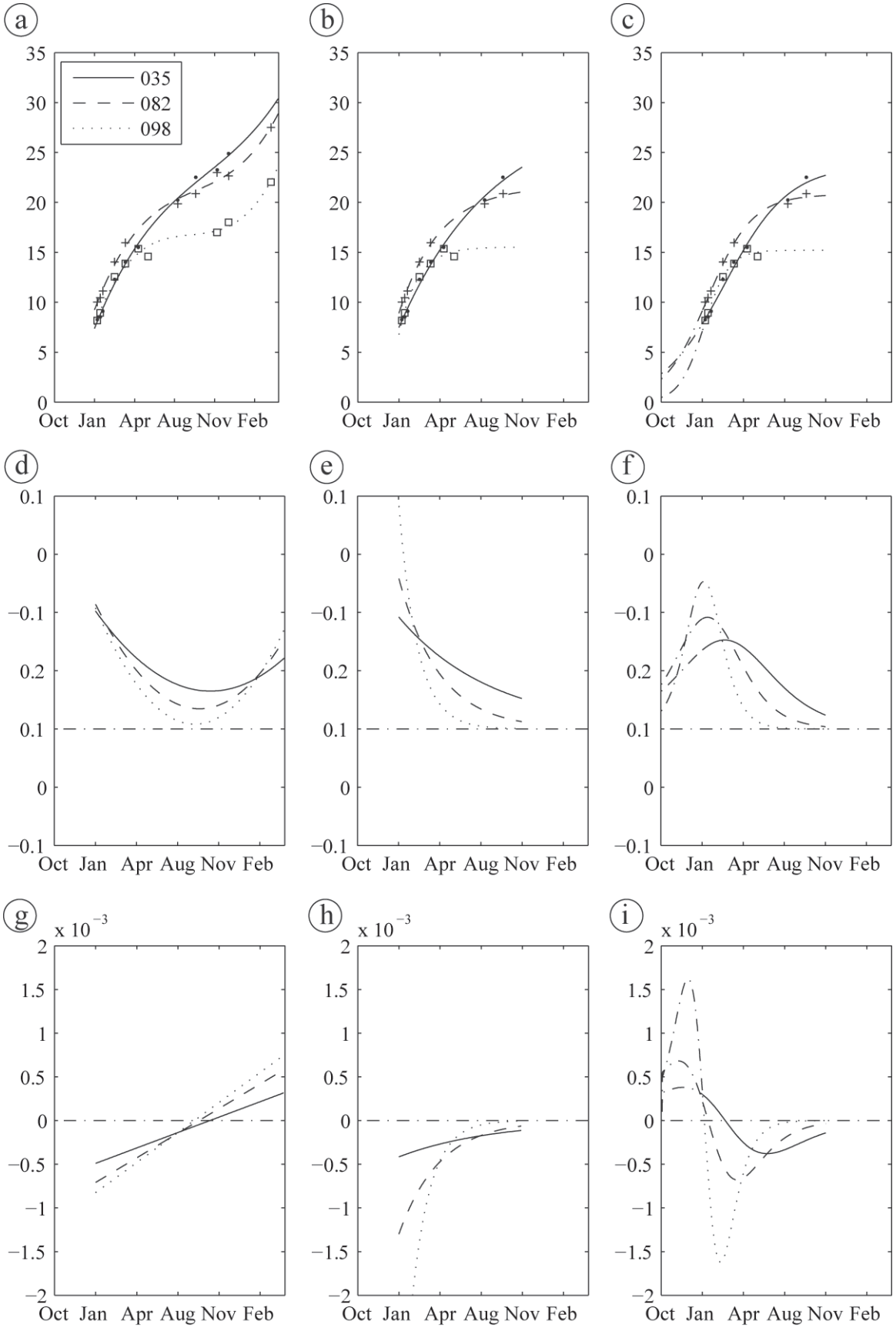
As explained above, 95 percent of the snails exhibit a decrease in growth rate from 100 to 350-450 *DAH*. Then, a fitting over this restricted period of time can be performed by a Von Bertalanffy curve (Von Bertalanffy, 1938) (Fig. 4b):

$$Y = b_1 - (b_1 - b_2)\, e^{-b_3 X}$$

where Y is a measure of size and X is the number of days between 100 and 400 *DAH*, b_1 is the asymptotic size, b_2 is the initial size (at 100 *DAH*) and b_3 is the intrinsic growth rate[13].

13 Note that the intrinsic growth rate (b_3) is a scalar that describes the rate of decrease in the instantaneous growth rate (dY/

Fig. 4: Growth curves of shell length (*SL* in mm) *versus* time and their first and second derivatives, using different fitting equations, for snails 035 (dots, solid line), 082 (crosses, dashed line) and 098 (squares, dotted line) raised in the same tank (*tank 1*). **a**: polynomial fitting between 100 and 560 *DAH*. **b**: Von Bertalanffy's fitting between 100 and 400 *DAH*. **c**: Verhulst's fitting between 0 and 400 *DAH*. Dashed dot lines represent the portion of the curve where no data is available (0-100 *DAH*). **d, e, f**: first derivatives of **a, b** & **c**, respectively. **g, h, i:** second derivatives of **a, b** & **c**, respectively. Note that T_{min} (**d**, **g**) and T_{max} (**f**, **i**) increase as the growth curves are flattened (from snails 098, 082, 035). GR_{min} (**d**) and GR_{max} (**f**) are negatively correlated: snail 035 has the highest GR_{min} and the lowest GR_{max}.


a
b
c
035
082
098
Oct Jan Apr Aug Nov Feb
d
e
f
g
h
i
x 10 −3

For the fitting to converge, the parameters were constrained to lie:

- between 5 and 60 mm for asymptotic size (b_1) when *SL*, *SW* or *CS* were taken as the measure of size. 5 mm is the size at 400 *DAH* of the smallest snail in the sample. 60 mm represents the average size found in nature for 3 years-old snails in this species [14] (Vasconcelos *et al.,* 2006).

- between 2 and 15 mm for b_2 when *SL* was taken as the measure of size; 1.5 and 10 for *SW*; and 2 and 13 for *CS*. The same constraints have been applied in polynomial fitting for the corresponding parameter p_4.

- between 0 and 0.025 for the intrinsic growth rate (b_3). It means that instantaneous growth rate is 'forced' to decrease from 100 to 400 *DAH*.

With this fitting, we can expect a continuous variation from a slowly decreasing growth rate (low b_3, high b_1) to a rapidly decreasing growth rate (high b_3, low b_1). As expected, the parameter b_3 is correlated with all four parameters of polynomial fitting; positively with p_1, p_3 & p_4 and negatively with p_2 (see Table 5 in appendices). Mean growth rate (*MeanGRB*) is measured as the area below the growth rate curve obtained by deriving the Von Bertalanffy curve between 100 and 400 *DAH* (Fig. 4e). The medians of *MeanGRB* are significantly different among tanks (Chi-sq = 12.62, p = 0.014, Table 1), the snails in *tank 1* tending to have a higher *MeanGRB* (median 0.04 mm *SL* / day or 1.2 mm *SL* / month) over this period of time than snails in *tanks 4 & 6* (median 0.034 mm *SL* / day or 1.02 mm *SL* / month) (Fig. 5e). But as seen above, snails of *tank 1* are those that exhibit the later (about 342 *DAH*) and longer quiescent phase (until 100 days). Most of snails in *tank 1* do not significantly resume growth before November-December 2003.

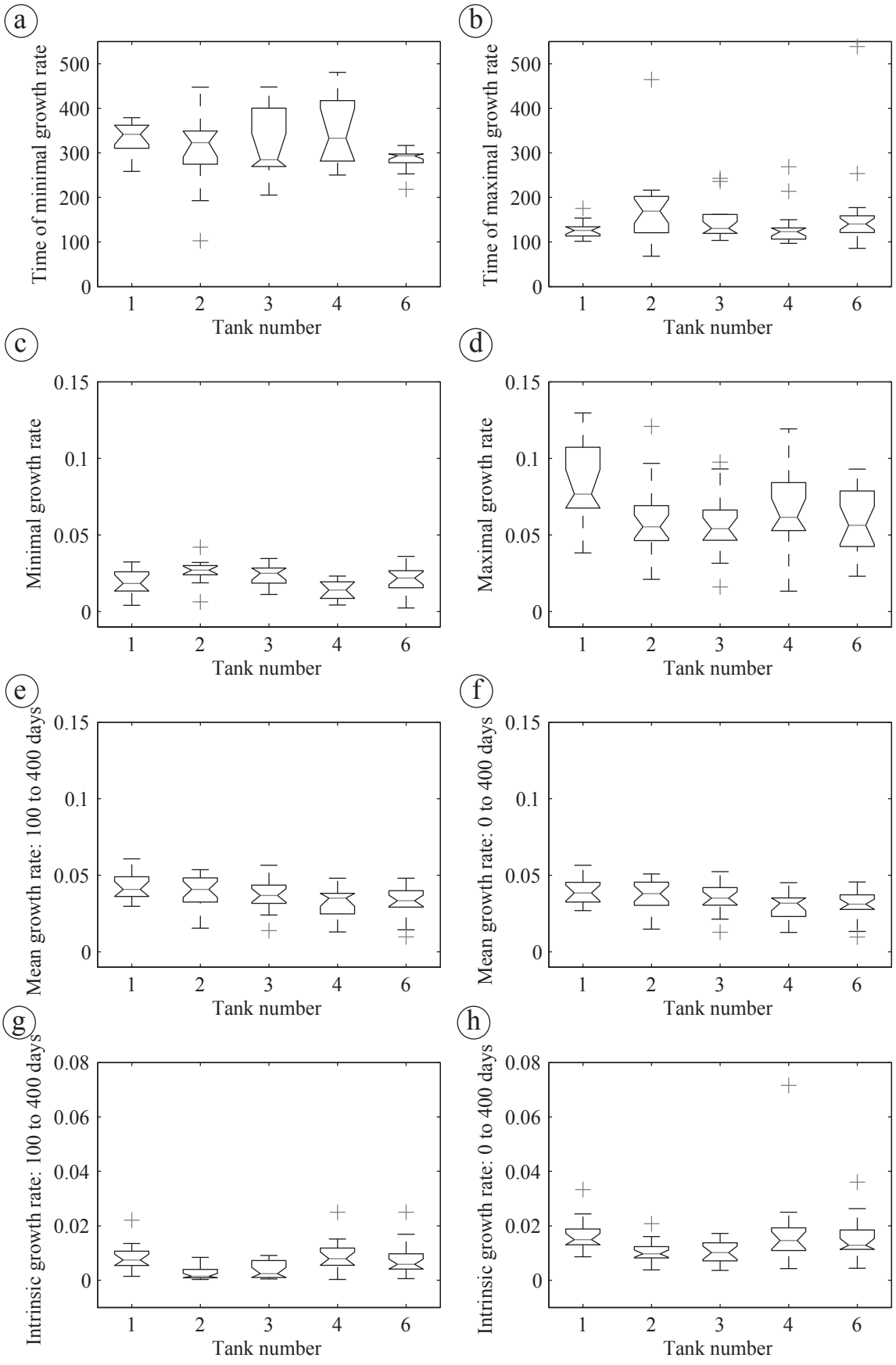
The parameter b_3 is also significantly different among tanks (Fig. 5g, Chi-sq = 24.97, p = $5e^{-5}$, Table 1), the snails of *tanks 1, 4 & 6* tending to have a higher b_3 than snails of *tanks 2 & 3*. It means that the growth rate of snails bred in *tanks 1, 4 & 6* tends to decrease faster than those of *tanks 2 & 3*.

Indeed, the polynomial fitting revealed that the proportion of poly1, poly2 & poly3 fittings was also different among tanks. There is about 5 percent of 'linear' growth curves in *tanks 1 & 6*, 10 percent in *tank 4*, 40 percent in *tank 2* and 45 percent in *tank 3*. There is no poly2 growth curve in *tank 3*, 4 percent in *tank 2*, 14 percent in *tank 6*, 33 percent in *tank 1* and

dt). Intrinsic growth rate and instantaneous growth rate should not be confounded here. The intrinsic growth rate (b_3) describes the 'shape' of the growth curve (size *vs.* time) and the 'shape' of growth rate curve (instantaneous growth rate *vs.* time). As shown by the Von Bertalanffy's equation, the instantaneous growth rate (dY/dt) is related to the intrinsic growth rate (b_3), but is also dependent on the initial and asymptotic sizes (b_2 and b_1), so that equating b_3 and instantaneous growth rate (or mean growth rate) is really hazardous. In chapters 3 & 4, instantaneous growth rate was unambiguously related to the intrinsic growth rate (called growth rate parameter) because no variation in initial/final size was assumed.

14 *H. trunculus* can exhibit an adult size of 80 mm. To constrain the asymptotic size to 60 mm or 80 mm does not affect our fitting results, because most snails in our sample exhibit a really smaller asymptotic size (15-25 mm). Only the most 'linear' growth curves do attain an asymptotic size of 60 or 80 mm; in these cases, the fittings are not improved by changing this parameter to lower or higher values.

Fig. 5: Comparison of growth parameters obtained by fitting *SL* against time using different fitting equations in tank replicates. **a**: time of minimal growth rate (T_{min})obtained by fitting the growth curves with a third degree polynomial equation. **b**: time of maximal growth rate (T_{max}) obtained by fitting the growth curves with a Verhulst's equation. **c**: minimal growth rate (GR_{min}) corresponding to T_{min} in **a**. **d**: maximal growth rate (GR_{max}) corresponding to T_{max} in **b**. **e**: mean growth rate (area below the first derivative of Von Bertalanffy's growth curve, *MeanGRB*) between 100 and 400 *DAH*. **f**: mean growth rate (area below the first derivative of logistic growth curve, *MeanGRL*) between 0 and 400 *DAH*. **g**: intrinsic growth rate (b_3, Von Bertalanffy's equation) between 100 and 400 *DAH*. **h**: intrinsic growth rate (l_3, Verhulst's equation) between 0 and 400 *DAH*. **Grey line**: median. **Box**: lower and upper quartile. **Whisker**: extend of the rest of data. **Crosses**: outliers. **Notches**: robust estimate of the uncertainty about the medians for box-to-box comparisons. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 5% significance level.


a
Time of minimal growth rate
0
100
200
300
400
500
1
2
3
4
6
Tank number
b
Time of maximal growth rate
0
100
200
300
400
500
1
2
3
4
6
Tank number
c
Minimal growth rate
0
0.05
0.1
0.15
1
2
3
4
6
Tank number
d
Maximal growth rate
0
0.05
0.1
0.15
1
2
3
4
6
Tank number
e
Mean growth rate: 100 to 400 days
0
0.05
0.1
0.15
1
2
3
4
6
Tank number
f
Mean growth rate: 0 to 400 days
0
0.05
0.1
0.15
1
2
3
4
6
Tank number
g
Intrinsic growth rate: 100 to 400 days
0
0.02
0.04
0.06
0.08
1
2
3
4
6
Tank number
h
Intrinsic growth rate: 0 to 400 days
0
0.02
0.04
0.06
0.08
1
2
3
4
6
Tank number

35 percent in *tank 4*. Clearly, *tanks 2 & 3* are characterized by poly1 or poly3 growth curves, whereas *tanks 1, 4 & 6* are characterized by poly2 and poly3 growth curves.

The differences in the proportion of poly1 and poly2 growth curves among tanks do not reflect differences in the density of data. Low frequencies of poly1 and high frequencies of poly2 are found in *tanks 1 & 4* with an average of 8 and 6 measurements per snail respectively, and the reverse is found in *tanks 2 & 3* with an average of 6 and 7 measurements per snail, that is irrespective of the number of measurements per snails. Von Bertalanffy growth curves also point out that growth rates in *tanks 2 & 3* tend to decrease more slowly than in other tanks, so the high frequency of poly1 (and respectively low frequency of poly2) in these tanks should be accounted for by other factors than differences in density of data or lack of data after 400 *DAH*.

Although we have no data[15] before January 15th 2003, it is tempting to try to obtain a growth curve starting from the presumptive date of hatching t_0. What is known is that shell length at hatching is 1.64 ± 0.25 mm (see below). Because of the incertitude on the precise date of hatching for each snail (77-117 before the 15th January 2003), size at t_0 (7th October 2002) is constrained between 0 and 5 mm.

First, a Von Bertalanffy's equation has been fitted over the 0-400 period of time with b_1 constrained between 5 and 60 mm (as above), b_2 between 0 and 5 mm and b_3 positive. But this fitting provided a size at t_0 (b_2) near zero for the snails with the smallest *IDs*, that is snails which had already hatched at t_0. If the growth rate had really decreased from hatching to 400 *DAH* ± 20 days, we would have expected the snails hatched before t_0 to have a size at this time greater than the hatching size (or at least equals to the higher bound of the observed embryonic shell sizes ≈ 2mm). An exception is for the first hatched snails which are well fitted by a poly1 growth curve and whose fitted size at t_0 could be found to be superior or equal to 2 mm. But to the exception of these snails, the fitting of Von Bertalanffy's equation over 0-400 days with the above constraints is worst than the same fitting over 100-400 *DAH* (the average over the 92 fitted growth curves of the root mean squared error is 0.49 and 0.35 respectively). So, we are let to assume that growth rate should increase over the period where we have no data, perhaps to the exception of the snails well fitted by a poly1 growth curve (then, their growth rate could have remained nearly constant over 0-560 *DAH*). Moreover, as described above, a small proportion of snails (5 percent) seemed to experience an increase in growth rate after the time of measurements (small but negative p_1).

(c) Verhulst's equation

If growth rates are assumed to increase before 100 *DAH* and decrease after that (at least until 400 *DAH*), we have to fit the growth rate curves over 0 to 400 *DAH* by a bell shaped growth rate curve. Then, the growth curve would have an inflexion point. The most frequently used growth curve of this type is the Verhulst's equation (Verhulst, 1838), also known as the logistic curve (Fig. 4c):

$$Y = l_1 \times l_2 / [(l_1 - l_2) e^{-l_3 X} + l_2]$$

where Y is a measure of size and X is the number of days between 0 and 400 *DAH*, l_1 is the asymptotic size, l_2 is the initial size (at t_0) and l_3 is the intrinsic growth rate[16].

15 Except measurements of embryonic shell, like width, spiral length, shell length, number of whorls, see below.

16 The same comments apply to the intrinsic growth rate

For the fitting to converge, the parameters were constrained to lie:

- between 5 and 60 mm for asymptotic size (l_1) when *SL*, *SW* or *CS* were taken as the measure of size (same constraints as b_1).

- between 0 and 5 mm for size at t_0 (l_2) when *SL, SW or CS* were taken as the measure of size .

- between 0 and 0.08 for the intrinsic growth rate (l_3).

At the inflexion point, the growth rate is maximal (Fig. 4f). The time of maximal growth rate (T_{max}) is the time at which the second derivative of the logistic equation is null (Fig. 4i). T_{max} is given by:

$$T_{max} = 1 / (l_3 \times \log [(l_1 - l_2) / l_2])$$

The growth rate at that time (GR_{max}) is:

$$GR_{max} = l_3 \times l_1 / 4;$$

The logistic equation imposes some constraints on the size at T_{max}: it is half of the asymptotic size (l_1). We imposed no other constraints upon the parameters l_1, l_2, and l_3 for T_{max} to be between 0 and 400 *DAH* [17].

The intrinsic growth rates of Von Bertalanffy' and Verhulst's equations are well correlated (Rho = 0.96, p = 0; see Table 5 in appendices). The parameter l_3 is significantly different among tanks (Chi-sq = 22.27, p = 1.7e-4, Table 1). The snails of *tanks 1, 4 & 6* have a higher l_3 than snails of *tanks 2 & 3* (Fig. 5h). The higher the l_3, the steeper the change in growth rate over time (Figs. 4c & f). Thus, the results of Verhulst's fitting are concordant with the polynomial and Von Bertalanffy's fittings which revealed that the proportion of poly1 growth curves ($p_1 = 0$, low b_3, low l_3) in *tanks 2 & 3* was higher than that in *tanks 1, 4 & 6* (Figs. 4g, h).

In 5 percent of the fittings, T_{max} is found to be superior to 400 *DAH*. It corresponds to the 5% of snails whose growth rate seems to increase between 0 and 400 *DAH* (snail031 in tank 3, snail064 in tank 2, snail097 in tank 1, snail112 in tank 2, snail134 in tank 6, see Appendices). The four last snails are fitted by a poly3 and T_{min} is evaluated between 100 and 220 *DAH*. It is considerably less than the T_{min} found for other poly3 growth curves (≈ 320 *DAH*). It cannot be deciphered whether these snails rather exhibit a slowly increasing growth rate (negative p_1), a nearly constant growth rate (p_1 null, low b_3, low l_3) or a rapidly and shortly decreasing growth rate followed by a slowly increasing growth rate as the third degree polynomial fitting tends to point out when p_1 is constrained to be positive.

These 'outliers' put apart, the differences in the medians of T_{max} among tanks is at the limit of being significant (Chi-sq = 9.5, p = 0.05, Table 1). The snails in *tanks 2, 3 & 6* tend to have a slightly delayed T_{max} compared to snails of *tanks 1 & 4* (median is 146 and 124 *DAH* respectively, Fig. 5b). Indeed, among the snails fitted by a poly3, T_{max} and T_{min} are inversely correlated (rho = -0.2713, p = 0.03).

Similarly, a negative correlation between GR_{min} and GR_{max} is to be expected (rho = -0.2853, p = 0.02). The lowest GR_{min} (0 mm *SL* / day) and the highest GR_{max} (0.1 mm *SL* / day or 3 mm *SL* / month) correspond to the snails which exhibit the most evident quiescent phases (high p_1, high b_3, high l_3). The snails that seem to grow more

of the Verhulst's equation and Von Bertalanffy's equation. See footnote 13. In the case of logistic fitting, however, the intrinsic growth rate (l_3) describes the rate of increase/decrease in instantaneous growth rate before/after the inflexion point respectively. It is thus a scalar that roughly describes the 'aggressiveness' of the growth curve. A high l_3 results in a rapidly increasing and subsequently rapidly decreasing instantaneous growth rate. As explained below, the inflexion time (T_{max}) is dependent on all three parameters l_1, l_2 & l_3. Thus, a high l_3 will be linked with an earlier inflexion time than a low l_3 (l_1 & l_2 being equal).

17 As a consequence, for growth curves well fitted by a poly1 equation, T_{max} can be found outside the range of data.

linearly (low p_1, low b_3, low l_3) have a quasi constant growth rate, and consequently GR_{min} (0.04 mm *SL* / day or 1.2 mm *SL* / month) and GR_{max} (0.05 mm *SL* / day or 1.5 mm *SL* / month) are nearly equal and are frequently (but not always) of intermediate value. Not surprisingly, low GR_{min} are found in *tanks 1 & 4* (median: 0.018 mm *SL* / day or 0.54 mm *SL* / month) and high GR_{min} in *tanks 2 & 3* (median: 0.027 mm *SL* / day or 0.81 mm *SL* / month) (Fig. 5c). Reciprocally, high GR_{max} are found in *tanks 1 and 4* (median: 0.08 mm *SL* / day or 2.4 mm *SL* / month) and low GR_{max} in *tanks 2 & 3* (median: 0.05 mm *SL* / day or 1.5 mm *S L* /month) (Fig. 5d). The medians of GR_{max} are significantly different among tanks (Chi-sq = 13.66, p =0.008, Table 1). In *tank 1*, GR_{max} is superior to that in other tanks. GR_{max} in *tank 4 & 6* is not significantly different from *tanks 2 & 3*. This is explained by the fact that snails in *tanks 4 & 6* tend to grow more slowly than in *tanks 1, 2 & 3*.

Mean growth rate (*MeanGRL*) is measured as the area below the derivative of the logistic curve. As expected, *MeanGRL* is highly correlated with *MeanGRB* (rho = 0.97, p = 0, and see Figs. 5e-f). *MeanGRL* is significantly different among tanks (Chi-sq =12.96, p = 0.01, Table 1), the snails bred in *tank 1* tending to grow faster in average than in *tanks 4 & 6* (until 400 *DAH*, Fig. 5f). Note that the GR_{max} (0.013 mm *SL* / day or 0.39 mm *SL* / month) of some particularly slowly growing snails can be 3 times lower than the GR_{min} (0.04 mm *SL* / day or 1.2 mm *SL* / month) of some rapidly growing snails (Figs. 5c-d). It seems that extensive variation in mean growth rates exist irrespective of the shape of the growth curves. For instance, *MeanGRB* or *MeanGRL* in *tanks 1 & 2* are nearly equal, whereas the former tank is characterized by poly3 growth curves and the latter by poly1 growth curves. To the contrary, *MeanGRB* (and *MeanGRL*) in *tanks 4 & 6* are significantly lower than in *tank 1* although the shape of the growth curves appears rather similar among these three tanks.

Note that l_1 and l_3 are negatively correlated (rho = -0.32, p = 0.002, see Table 5). The snails which exhibit the most linear growth curves (low l_3) tend to have a high asymptotic size, which is frequently near or equal to the upper bound of l_1 (60 mm). The same holds for the parameters b_1 and b_3 of Von Bertalanffy fitting (rho = -0.78, p = 0, Table 5). Given the slow rate at which growth seems to decrease for these snails, the asymptotic size would be unrealistically high if we had not put a few constraints on b_1 and l_1.

The parameters l_3 and l_2 are also correlated (rho = -0.64, p = 0, Table 5), pointing out that under the constraints of the logistic fitting, the snails with the more 'linear' growth curves tend to have a larger size at t_0. However, l_2 is negatively correlated with *ID*, in accordance with the expectations concerning the under/over-estimation of date of hatching (rho = -0.28, p = 0.0062).

In order to clarify this, we measured the size of the embryonic shell of the snails in our sample (92 snails). We found that the embryonic shell width is 0.95 ± 0.14 mm. From measurements of shell length and width on dead embryonic shells, we found that the corresponding embryonic shell length would be 1.64 ± 0.25 mm in our sample. This size estimation corresponds well to the range of embryonic shell length reported by Vasconcelos *et al.* (2004) who estimated shell length at hatching to be 1.64 ± 0.22 mm. The estimated values of initial size (l_2) using a logistic fitting are more variable than expected from our measurements of embryonic shell size. Also, the estimated initial sizes are over-estimated for

a number of snails. So, we tried to correct for the uncertainty of the date of hatching for each snail ($t_0 \pm 20$ *DAH*), using a different parameterization of the logistic equation:

$$Y = a_1 - a_1 / [1 + e^{a_2 \times (X + a_3)}]$$

where a_1 is the asymptotic size; a_2 is the intrinsic growth rate; a_3 is the onset of the real date of hatching relative to t_0. A positive/negative a_3 leads to a "post/pre-displacement" of the curve. X is taken between 100 and 400 *DAH*.

Using *SL* as the measure of size, we constrained a_1 to lie between 5 and 25; a_2 between 0 and 0.08 and *a3* between -147 and 107. Then, we searched for the time at which the fitted *SL* would correspond to the measured *SL* at hatching for each snail. If this value lay within $t_0 \pm 20$ *DAH* [a_3 between -117 and -77] the fitting would be improved (and the real date of hatching would be better approximated). However, this was only the case for the snails which were already well fitted by the previous parameterization of the logistic function. For the snails which tended to have a low l_3 ('linear' growth curve), the 'correct' size at hatching would correspond to about $t_0 - 120$, which is clearly impossible. This result illustrates that these snails grew faster from hatching to 100 *DAH* than what predicted by using the logistic fitting.

It is interesting to compare the evolution of shell length (*SL*) in the tank replica over time (Figs. 6-9). At t_0, snails in *tanks 2 & 3*, with a median *SL* of 3.7 mm and 4 mm respectively tend to be larger than in other tanks (Fig. 6a; Figs. 7a, c, e, g, i, Chi-sq = 21.74, p = 0.0002, see Table 2 in appendices). But 100 days later, snails in *tank 1* appear to be larger than in other tanks (Fig. 6b; Figs. 7b, d, f, h, j, Chi-sq = 16.45, p = 0.002, see Table 2). The situation remains in this state (Figs. 6c-e; Fig. 8) until 500 days where the size differences in *tanks 1, 2, 3 & 6* nearly vanish (Fig. 6f; Fig. 9, Chi-sq = 3.67, p = 0.45, see Table 2).

(2) Shape

Variation in shell shape is analysed by geometric morphometrics of landmarks based on the aperture and by a PCA on elliptic Fourier coefficients of apertural contour (Fig. 1A). The components obtained by the landmarks analysis are hereafter denoted by RW_1, RW_2, RW_3 & RW_4. The components obtained by elliptic Fourier analysis are denoted PF_1 & PF_2.

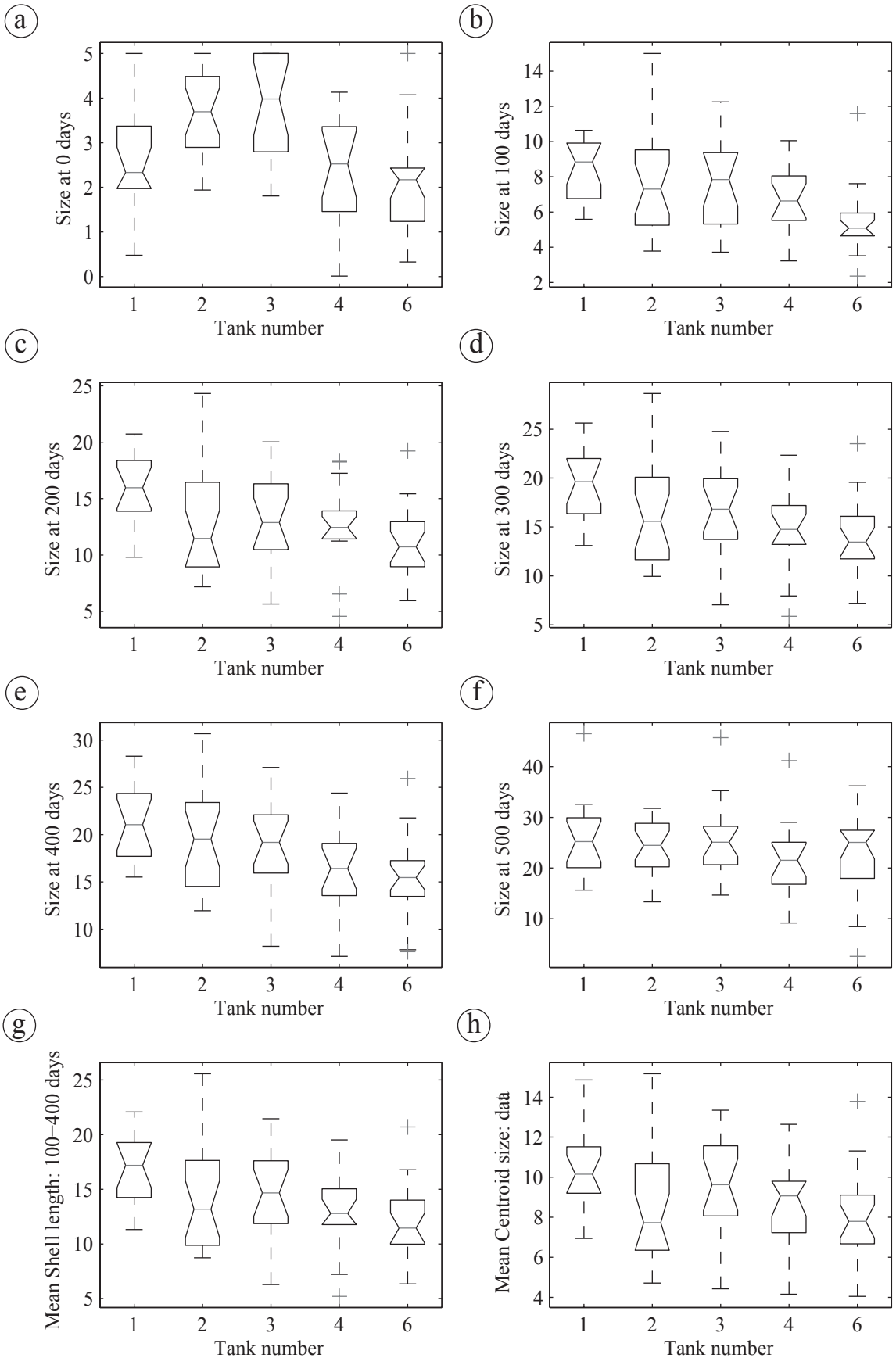
Figure 10 represents the percentage of variance explained by relative warps (Fig. 10a), principal factors based on 100 harmonics (Fig. 10b) and principal factors based on 10 harmonics (Fig. 10c). RW_1, RW_2, RW_3 & RW_4 account for 49, 23, 18 and 10 percent of the sample variance

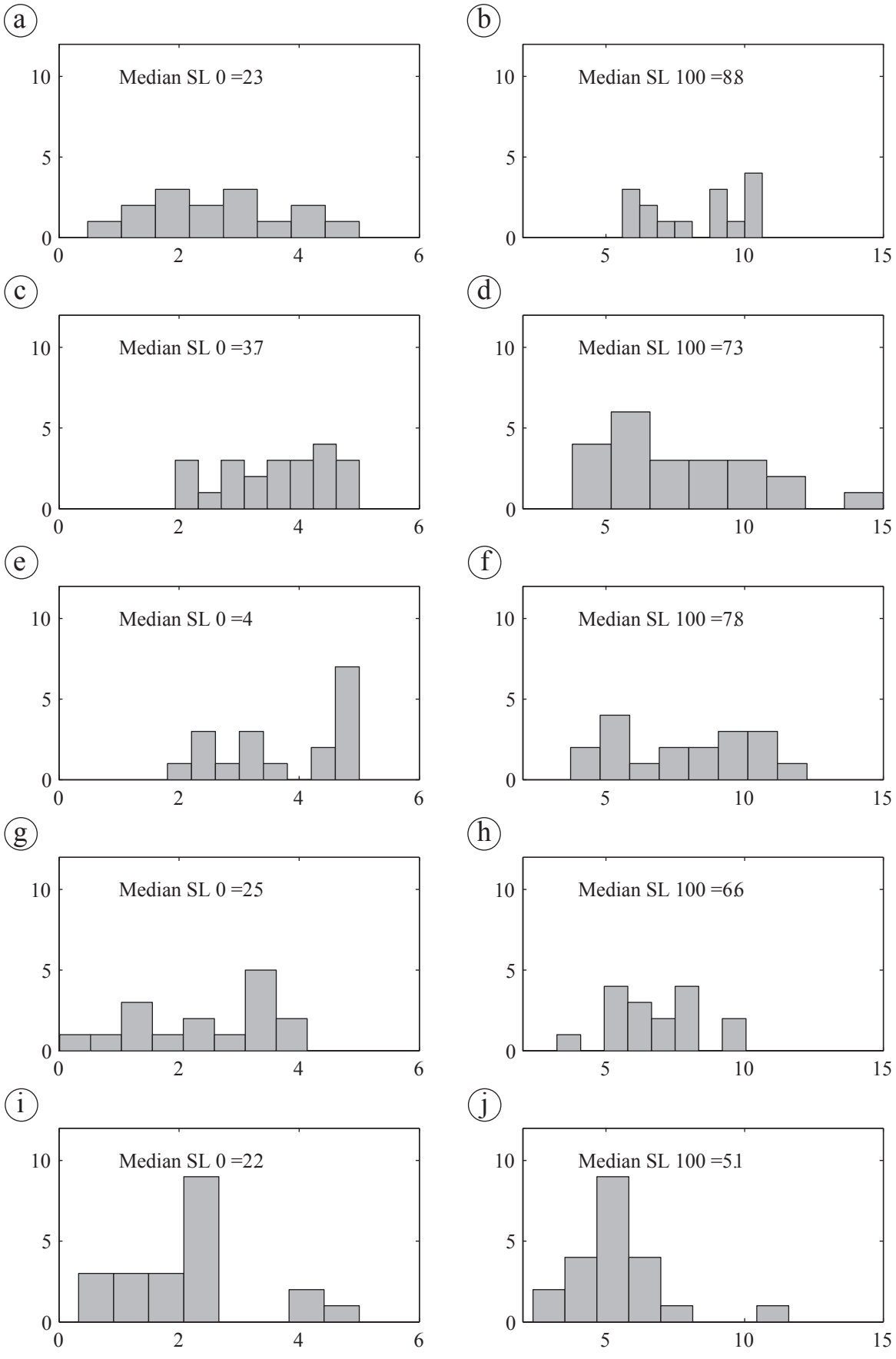
Fig. 6: Comparison of shell length (*SL*) in tank replicates. **a**: *SL* at t_0. **b:** *SL* at 100 *DAH*. **c**: *SL* at 200 *DAH*. **d**: *SL* at 300 *DAH*. **e**: *SL* at 400 DAH. **f**: *SL* at 500 *DAH*. **g**: Mean shell length (*MS*) over 100-400 *DAH*. **h**: Mean aperture centroid size over 100-400 *DAH* (using *CS*). **a**: Verhulst's equation. **b-e**, **g-h**: Von Bertalanffy's equation. **f**: polynomial equation. **Grey line**: median. **Box**: lower and upper quartile. **Whisker**: extend of the rest of data. **Crosses**: outliers. **Notches**: robust estimate of the uncertainty about the medians for box-to-box comparisons. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 5% significance level.

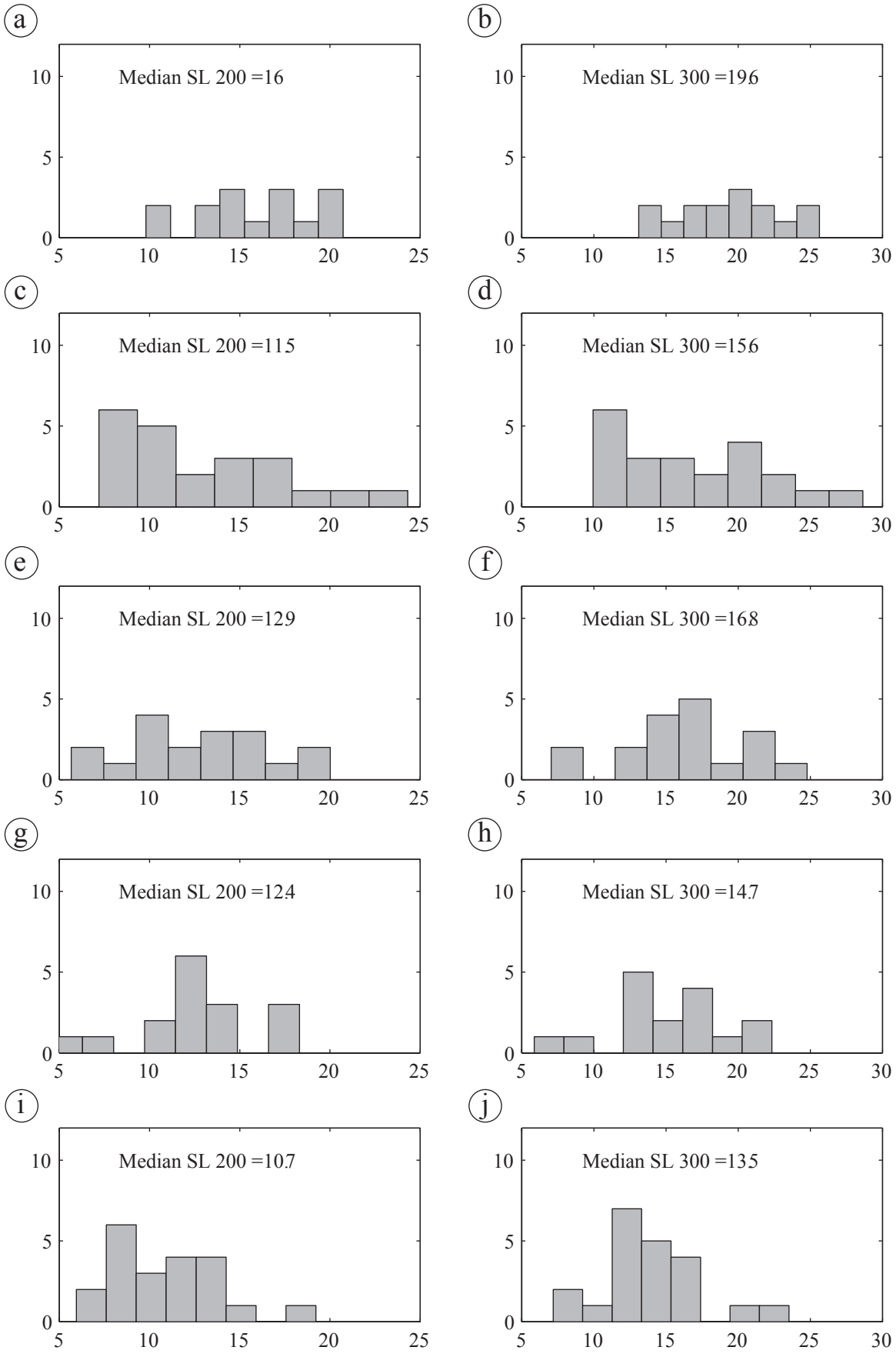
Fig. 7: Histograms of *SL* in tank replicates (lines) at t_0 (**a**, **c**, **e**, **g**, **i**) and 100 *DAH* (**b**, **d**, **f**, **h**, **j**). **First column**: Verhulst's equation. **Second column**: Von Bertalanffy's equation.

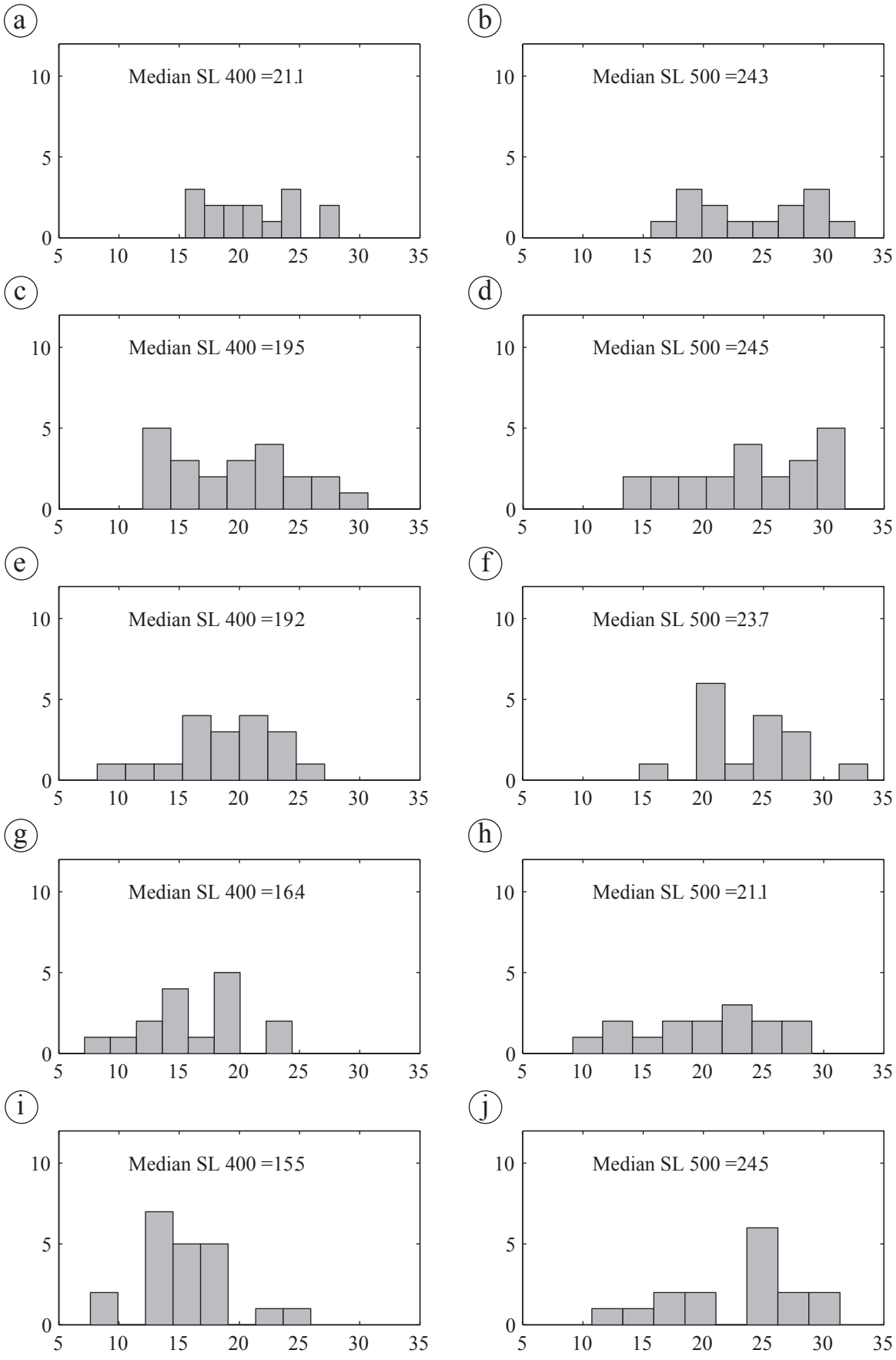
Fig. 8: Histograms of *SL* in tank replicates (lines) at 200 (**a**, **c**, **e**, **g**, **i**) and 300 *DAH* (**b**, **d**, **f**, **h**, **j**). Von Bertalanffy's equation.

Fig. 9: Histograms of *SL* in tank replicates (lines) at 400 (**a**, **c**, **e**, **g**, **i**) and 500 *DAH* (**b**, **d**, **f**, **h**, **j**). **First column**: Von Bertalanffy's equation. **Second column**: polynomial equation.


a
Size at 0 days
Tank number
b
Size at 100 days
Tank number
c
Size at 200 days
Tank number
d
Size at 300 days
Tank number
e
Size at 400 days
Tank number
f
Size at 500 days
Tank number
g
Mean Shell length: 100–400 days
Tank number
h
Mean Centroid size: data
Tank number


a
Median SL 0 =23
b
Median SL 100 =88
c
Median SL 0 =37
d
Median SL 100 =73
e
Median SL 0 =4
f
Median SL 100 =78
g
Median SL 0 =25
h
Median SL 100 =66
i
Median SL 0 =22
j
Median SL 100 =51


a
Median SL 200 =16
b
Median SL 300 =19.6
c
Median SL 200 =11.5
d
Median SL 300 =15.6
e
Median SL 200 =12.9
f
Median SL 300 =16.8
g
Median SL 200 =12.4
h
Median SL 300 =14.7
i
Median SL 200 =10.7
j
Median SL 300 =13.5


a
Median SL 400 =21.1
b
Median SL 500 =243
c
Median SL 400 =195
d
Median SL 500 =245
e
Median SL 400 =192
f
Median SL 500 =23.7
g
Median SL 400 =164
h
Median SL 500 =21.1
i
Median SL 400 =155
j
Median SL 500 =245
0
5
10
15
20
25
30
35

respectively. The first four components of elliptic Fourier analysis using 100 harmonics account for 73, 7, 4 and 2 percent of the sample variance respectively. With 10 harmonics, PF_1 and PF_2 account for 78 and 7 percent of the sample variance. By comparing the results of these two analyses, it appeared that 10 harmonics were sufficient to approximate the shell outline precisely enough. The following results are thus based on 10 harmonics.

Figures 11a-b display the four non-affine components of variation (RW_1, RW_2, RW_3 & RW_4) within two size categories[18]. Figure 11c presents PF_1 against PF_2 for the same size categories. In figure 11d, the two components of affine transformation of aperture shape are shown (hereafter U_1 & U_2). It is clear that RW_1, RW_2, PF_1, U_1 and U_2 are dependent on size. This is confirmed in figure 12, where each component has been regressed against *CS* (see Table 3 in appendices). A significant correlation is also found for RW_3 and PF_2. For each component, the 834 scores have also been regressed against time (Table 3).

The affine components U_1 & U_2 document an allometry of aperture which becomes relatively wider with size[19]. The axis of deformation is not really parallel to the *x* axis (aperture width), but slightly oriented to the posterior side (relative distance between *landmarks 2 & 5*). The orientation of affine dilatations is 22° and -68° along each component (counterclockwise).

U_1 & U_2 are correlated with *PF1* (Table 3). Along PF_1, the variation also concerns the relative wideness of the aperture (Fig. 13a). Large snails tend to have a high score on PF_1,

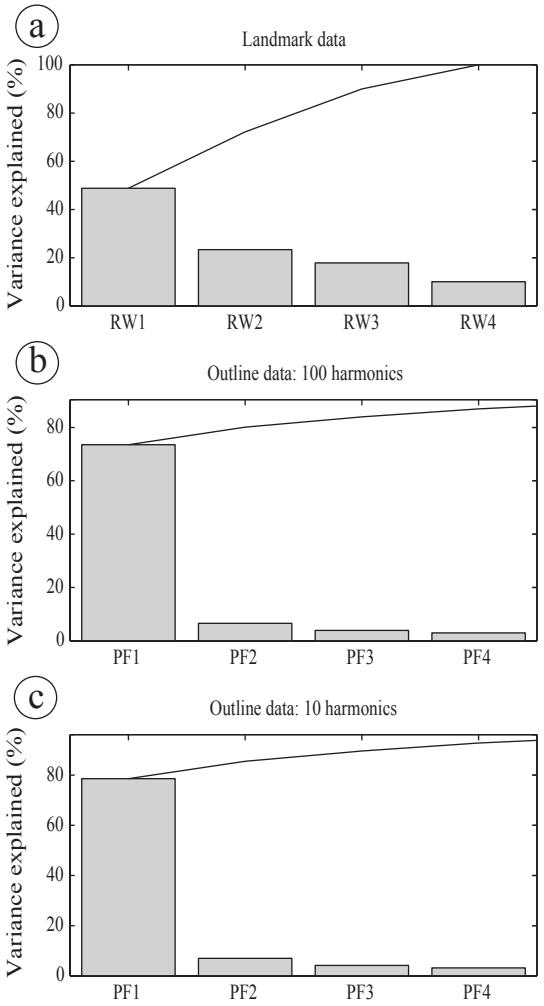


Fig. 10: Percent of variance explained by the first four components obtained by analyzing three data sets. **a**: landmark data (Fig. 2A). **b**: outline data (100 harmonics). **c**: outline data: 10 harmonics (Fig. 2B).

meaning that their aperture is relatively wider and their siphon is somehow shortened (Figs. 13a-b, red contour). RW_1 shows a similar pattern of variation, highlighting that the siphon tends to become relatively shorter over ontogeny (Figs. 14 a-c). Warp1 represents variation in the position of *landmarks 2-3-4*. Negative scores on this warp (Fig. 14a) correspond to a centripetal movement of *landmarks 2 & 3* (no spine, relatively long siphon), relative to the consensus (Fig. 14b). Positive scores (Fig. 14c) correspond to a centrifugal movement of *landmarks 2 & 3* (strong spine and relatively short siphon), relative to the consensus.

18 Relative contribution of each landmark: LM1: 40 %; LM2: 35 %; LM3: 15%; LM4: 5 %; LM5: 5%.

19 The uniform components have been treated separately from relative warps analysis (non-affine components). For this purpose, a routine in Matlab has been written, using the method described by Rohlf & Bookstein, 2003.

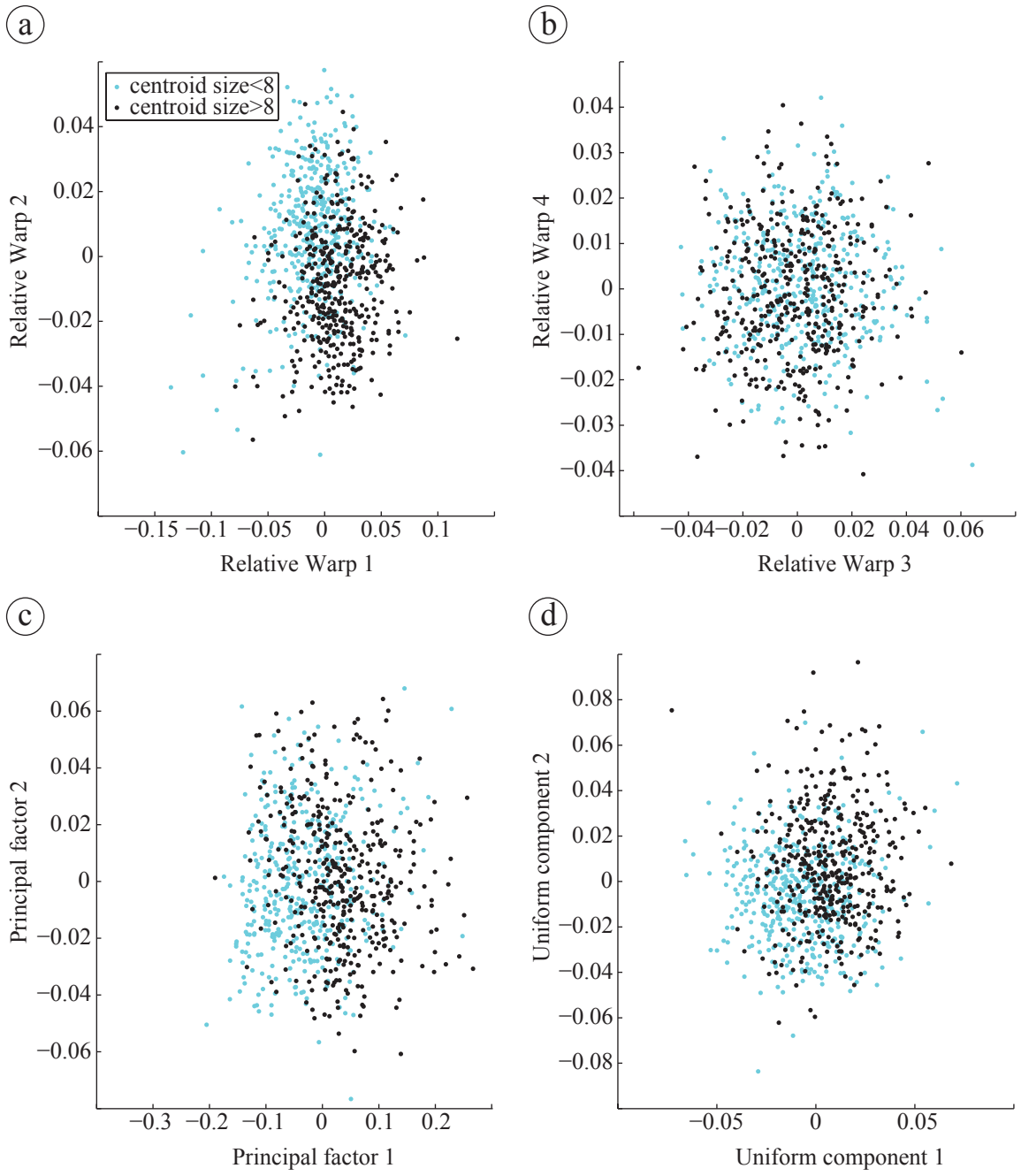


Fig. 11: **a-b**: first four relative warps components (*RW*) obtained from landmark data on the aperture (834 apertures). **c**: first two principal factors (*PF*) obtained from the PCA on the elliptic Fourier coefficients using10 harmonics (834 apertures). **d**: first two uniform components (*U*) obtained from landmark data (834 apertures). **a**: RW_1 against RW_2. **b**: RW_3 against RW_4. **c**: PF_1 against PF_2. **d**: U_1against U_2. Size categories are based on the median of the aperture centroid size (*CS*). **Blue**: small snails ($CS \leq 8$). **Black**: large snails ($CS > 8$).

The curvature of the aperture also tends to decrease with increasing scores on PF_1. This is in accordance with RW_2 and RW_3 which also suggest changes in aperture convexity (Fig. 14d-i). Indeed, it seems that RW_2 and RW_3 decompose the intensity of ornamentation along *x* and *y* coordinates: the distances between the umbilical suture (*landmark 1*) and the main spiral chord (*landmark 2*) along *x* and *y* tend to increase with size. Both distances tend to vary with aperture convexity, the spinier the aperture, the gentler the change in curvature around the siphon.

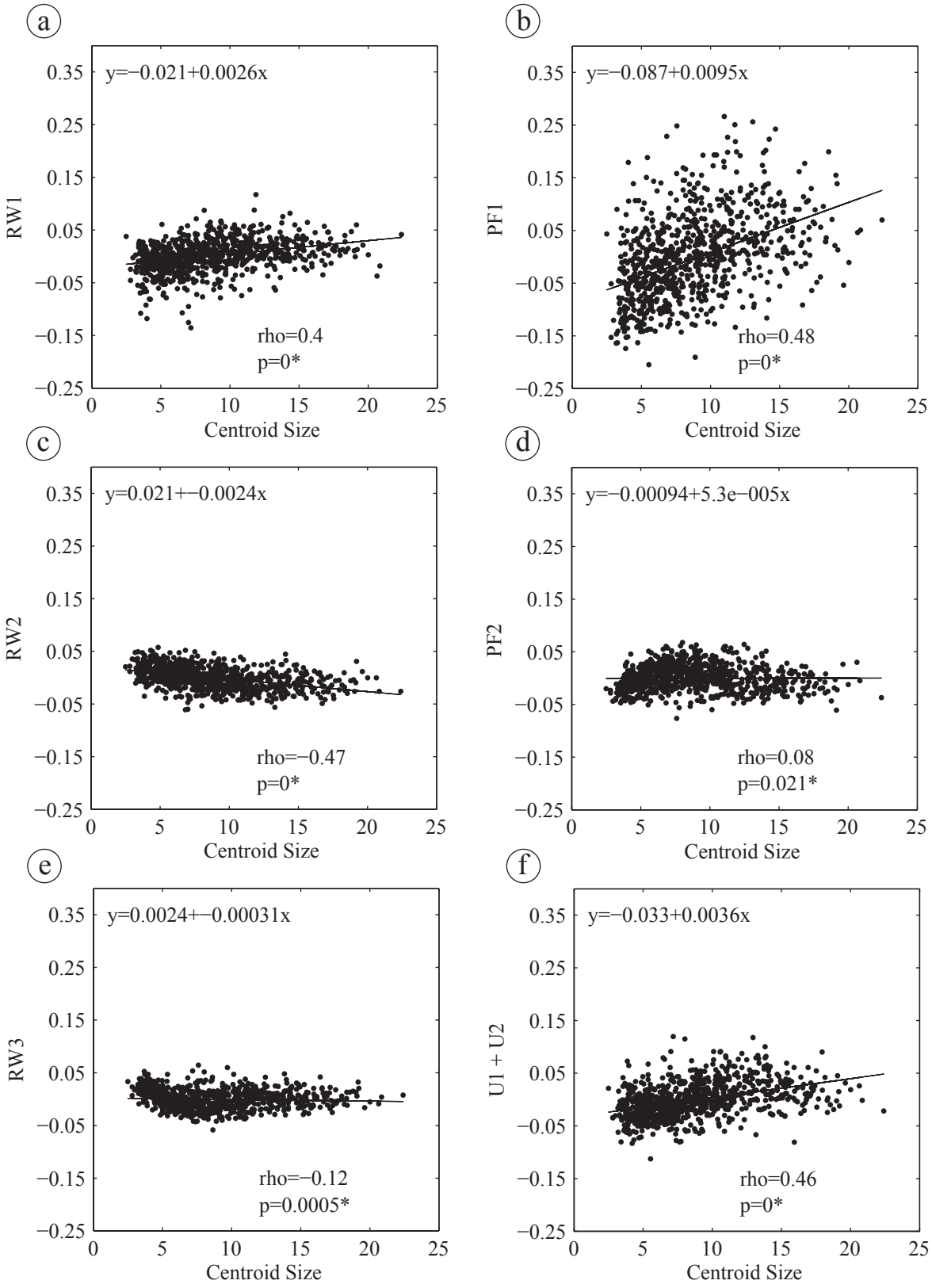


Fig. 12: Correlations between *RW* (landmark data, **first column**), *PF* (outline data, **b**, **d**) or U_1+U_2 (landmark data, **f**) and aperture centroid size (*CS*). Significant correlations are shown by an asterix and the corresponding regression lines using a robust fit (p-value<0.01; Spearman's *rho* indicates the correlation value). 834 apertures. *CS* is not normally distributed, so non-parametric tests were used (Pearson's *r* provided similar results).

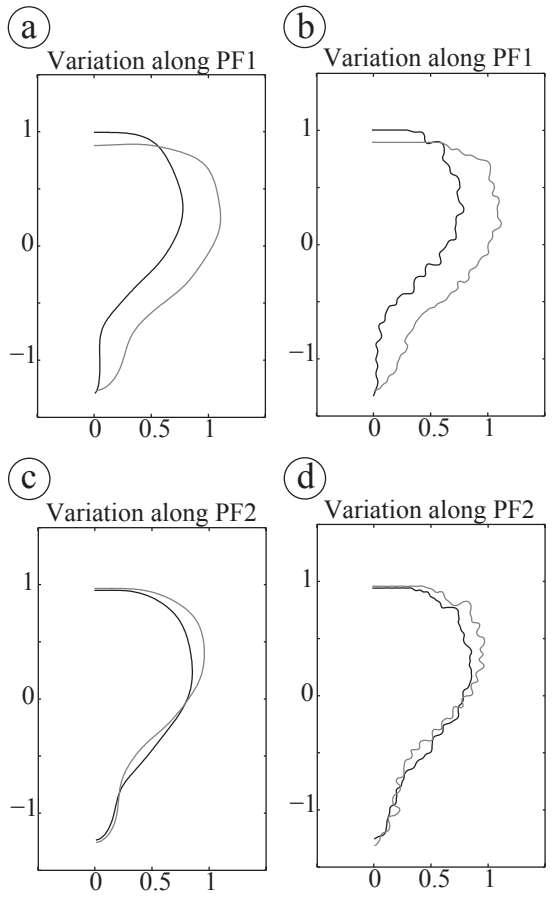


Fig. 13: Outline reconstruction of aperture using either 10 (**a**, **c**) or 100 harmonics (**b**, **d**). **a-b**: variation along PF1. **c-d**: variation along PF2. **Black/ grey**: negative/positive scores on principal factors, respectively.

PF_1 is correlated with both RW_2 and RW_3, though the former correlation is stronger (Table 3). RW_3 is more correlated with PF_2 than with PF_1 (Table 3). PF_2 describes changes that are localized to the posterior part of the aperture (Figs. 13c-d). This factor seems to be somehow related to the size of the spine on the main spiral chord (*landmark 2*).

The scores along U_1, U_2, RW_1 and PF_1 are consistent with a traditional morphometric analysis using the ratio between shell length and shell width to quantify gross shell morphology. The shell shape ratio (*SR*) is negatively correlated with *CS* and PF_1: shell width tends to increase faster than shell length (Table 3).

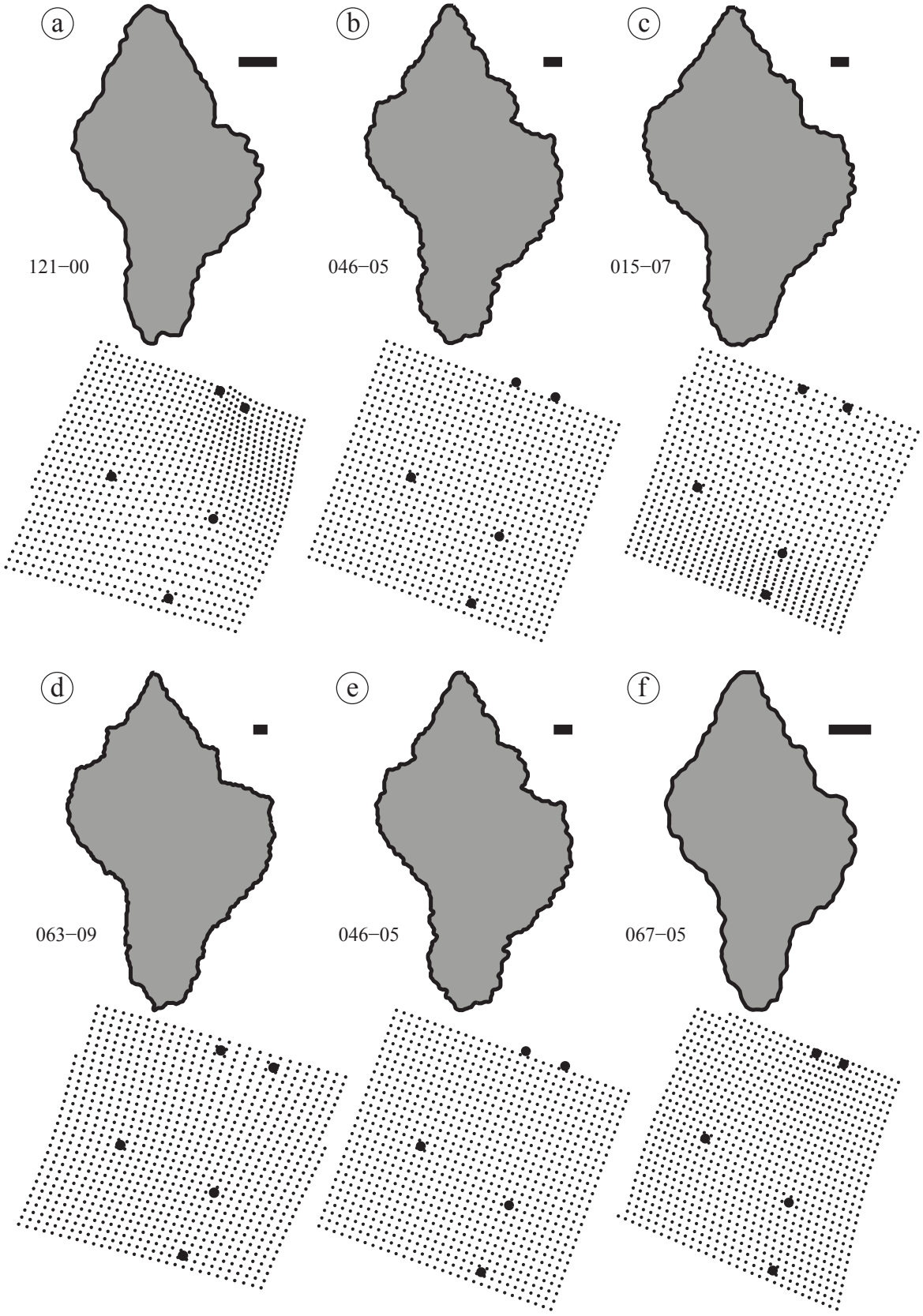
Elliptic Fourier analysis tends to condense on a single factor aspects of change in shape that seem different from the relative warp analysis (U_1, U_2, RW_1 & RW_2). The second principal component (PF_2, 7 % of variance explained) is related to RW_3 (Table 3).

To obtain the average scores per snail along each component, the scores corresponding to the same specimen were regressed against time, constraining the slope of the regression to be negative or positive according to the sign of the correlation between each component and time on the whole sample (Table 3, Plates in appendices). The average score for each specimen was then estimated as the area below the regression line between 100 and 400 *DAH* (MRW_1, MRW_2 & MRW_3). Similarly, the average shell length (*MS*) was estimated as the area below the growth curves fitted by the Von Bertalanffy equation over 100-400 *DAH*[20]. Figures 15a, c, e highlight that MRW_1 is positively correlated with *MS* whereas MRW_2 and MRW_3 are negatively correlated with *MS*. Interestingly, MRW_2 and MRW_3 are correlated (rho = 0.42, p = $3e^{-5}$), pointing out that among specimens these two factors vary accordingly.

Variation in the 'strength of ornamentation' (MPF_2, MRW_2, MRW_3) is related to the mean spacing between growth halts (*MGHS*), the snail with the more intense ornamentation tending to have more widely spaced growth halts (Figs. 16c-e). *MGHS* is also related to the aperture allometry documented by the uniform components (Fig. 16f). No significant correlation[21] is found between MPF_1 and *MGHS* (Fig. 16b), nor between MRW_1 and *MGHS* (Fig. 16a).

20 It has been checked that average sizes calculated as the mean of aperture centroid size (*CS*) for each snail and as the area below the growth curves in shell length (*MS*) were not significantly different. See also Figs. 6g-h

21 These correlations are marginally significant.


a
121–00
b
046–05
c
015–07
d
063–09
e
046–05
f
067–05

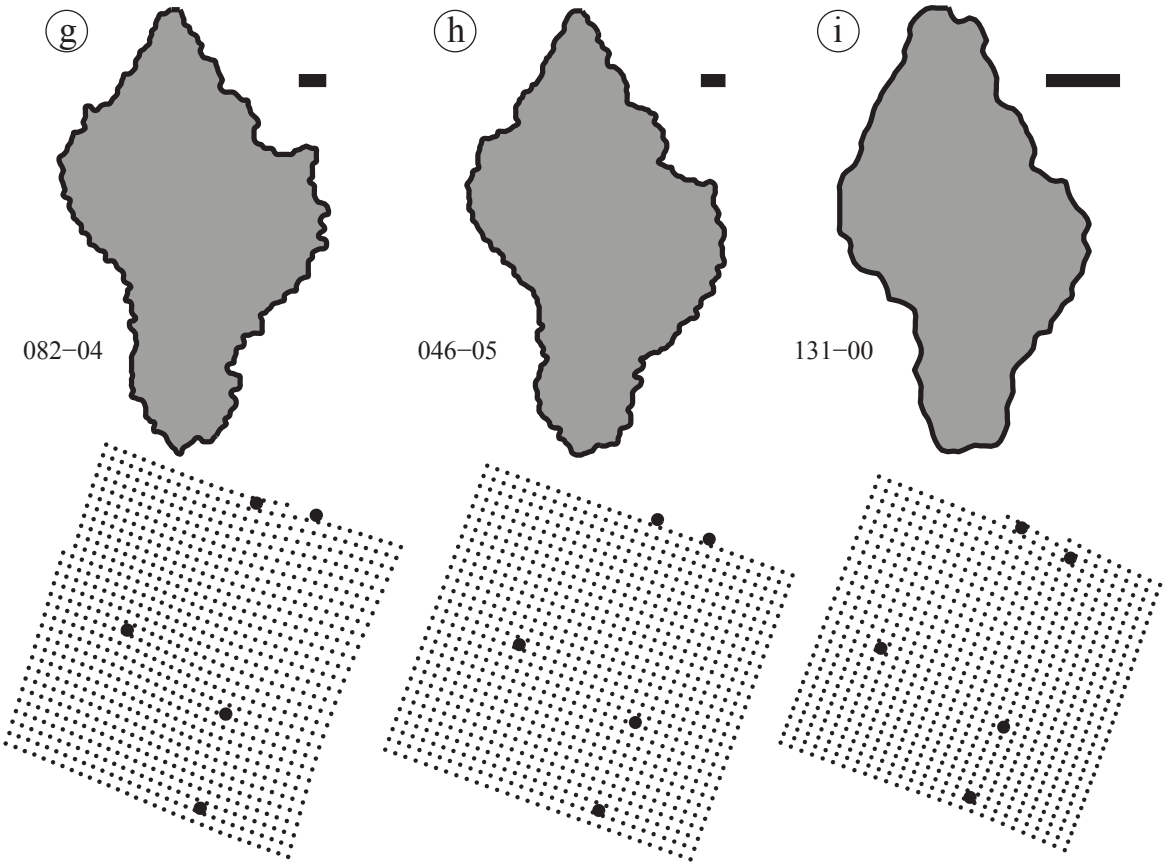


Fig. 14: Variation of aperture shape along the first three relative warps. **a-c**: RW_1; **d-f**: RW_2; **g-i**: RW_3. **Left**: negative score. **Center**: consensus. **Right**: positive score. The whole contour of shell shape of extreme variants on each component is shown along the corresponding thin-plate spline grids.

An overlook of shape variation in our sample is displayed in figure 17, with the shell contours of three variants redrawn at the same shell length at different times of ontogeny.

Results using traditional biometrics are generally consistent with those of geometric morphometrics. For instance, regressions of *SL* on *SW* illustrate that the wider variants are those that exhibit the largest *MGHS* and the most intense ornamentation (Figs. 18d-e). However, using such measurements somehow complicates the analysis, since *SW*, as measured here, may take into account the spines on the previous whorl if visible. However, whether spines on the previous whorl are visible or not depends on the spacing between growth halts. Such measurements can then artificially increase or decrease the variation between shells. Also, the changes in aperture shape are not easily retrieved from such global measurements, and may be completely missed.

(3) Growth rhythm, growth rate and shape

Up to 400 *DAH*, the mean number of growth halts per month (*MGHT*) seems to be related to the intrinsic growth rate of Von Bertalanffy's and Verhulst equations (b_3, l_3) obtained by fitting size against time: the snails displaying a high *MGHT* tend to have a more constant growth rate (low b_3, low l_3) over time and consequently a more linear growth curve (rho = -0. 25, p = 0.02 and rho = -0.2, p = 0.04, respectively, Figs. 18a-c, e).

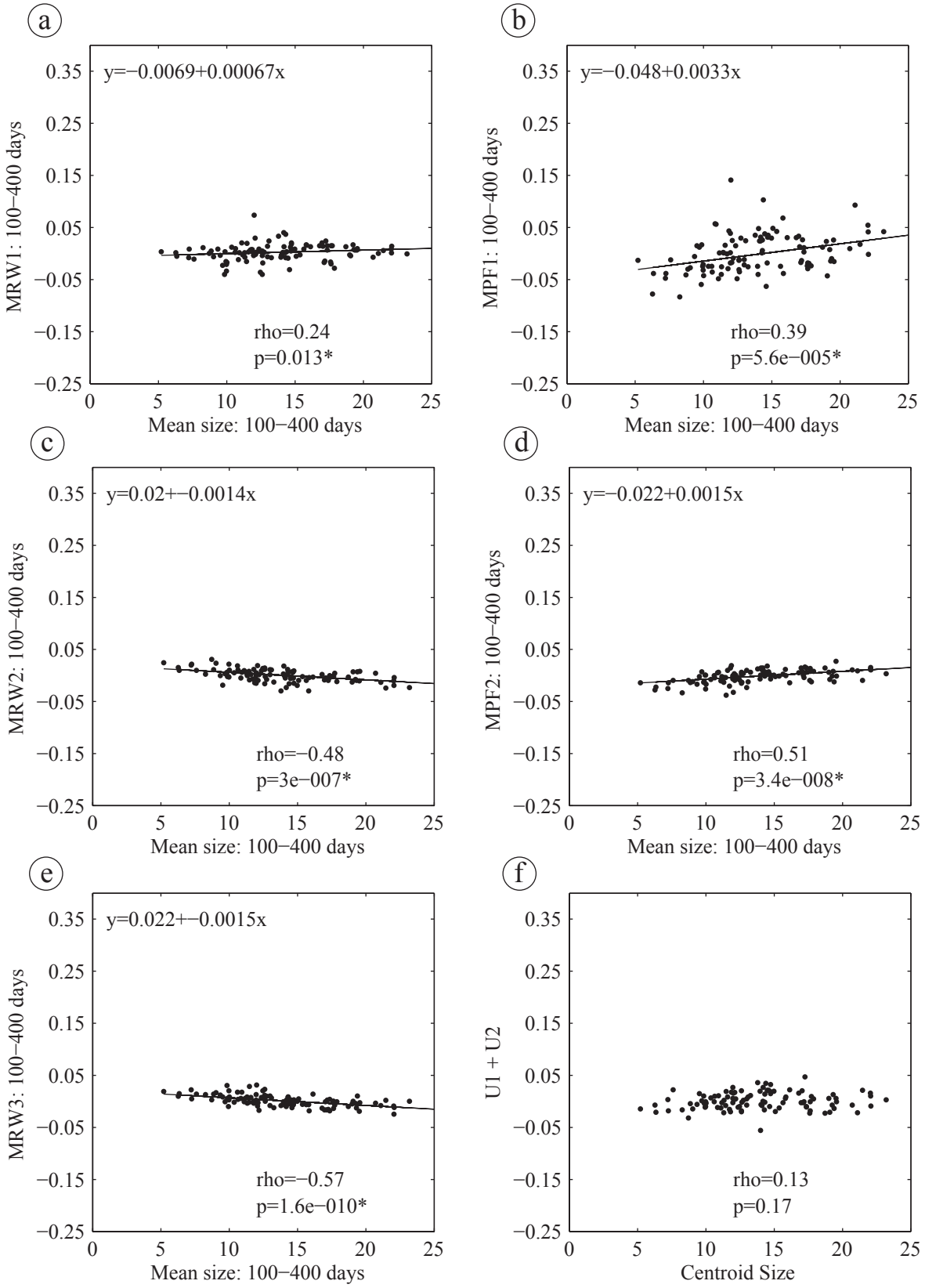


Fig. 15: Correlations between *MRW* (landmark data, **first column**), *MPF* (outline data, **b**, **d**) or $MU_1 + MU_2$ (landmark data, **f**) and mean aperture centroid size. Significant correlations are shown by an asterix and the corresponding regression lines using a robust fit (p-value<0.01; Spearman's *rho* indicates the correlation value). 151 snails.

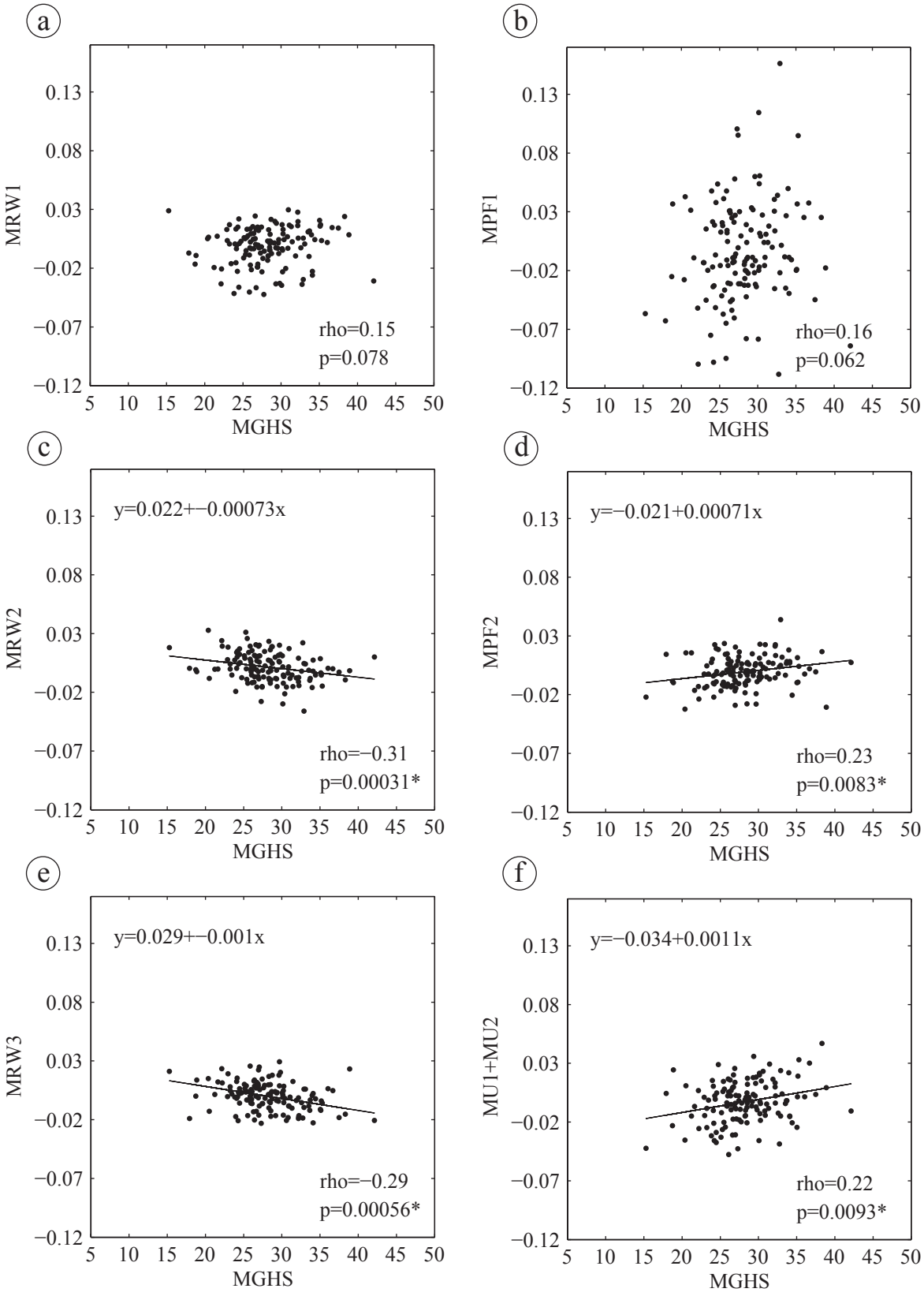


Fig. 16: Correlations between *MRW* (landmark data, **first column**), *MPF* (outline data, **b**, **d**) or $MU_1 + MU_2$ (landmark data, **f**) and mean spacing between growth halts (*MGHS*). Significant correlations are shown by an asterix and the corresponding regression lines using a robust fit (p-value<0.01; Spearman's *rho* indicates the correlation value). 92 snails.

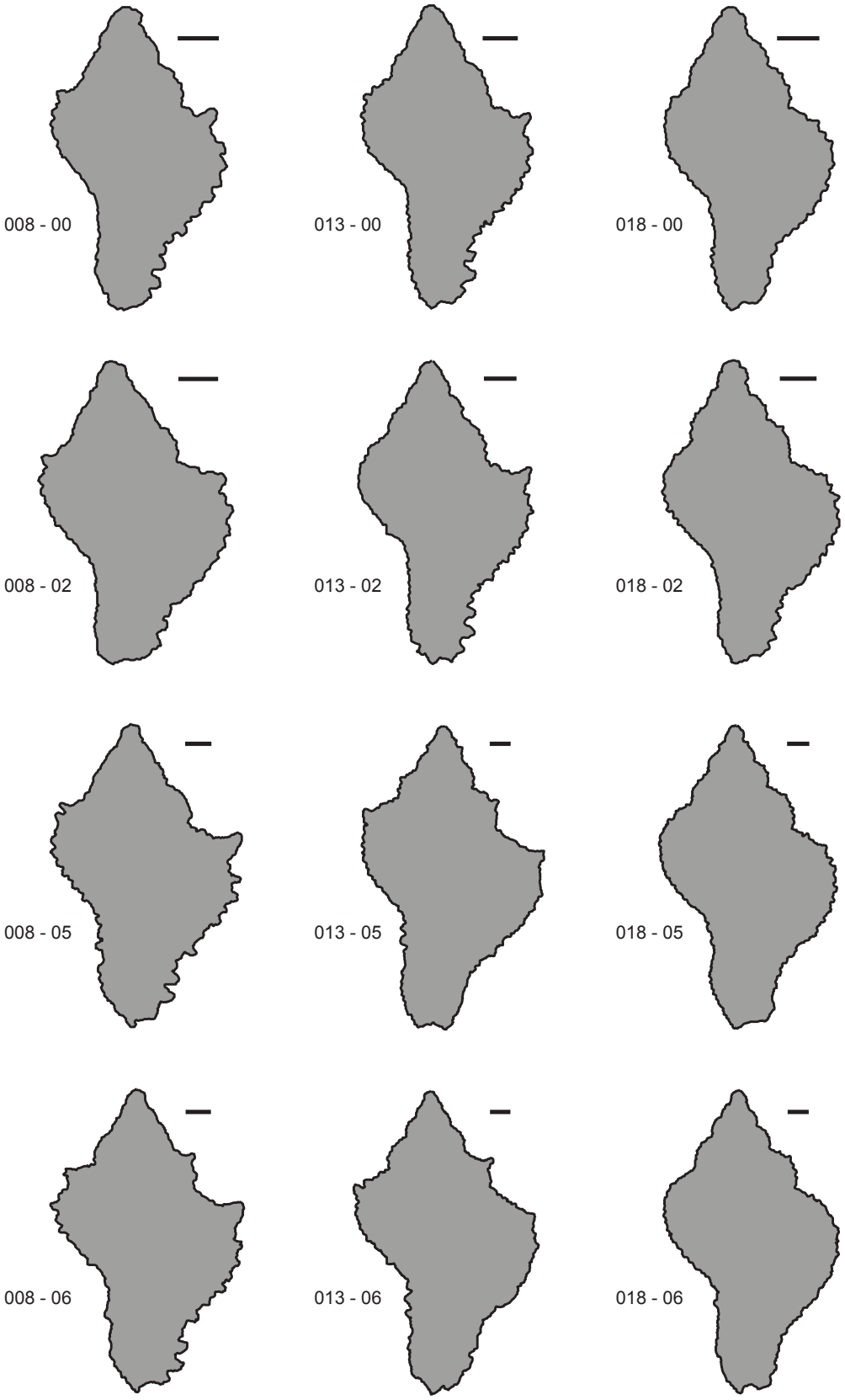
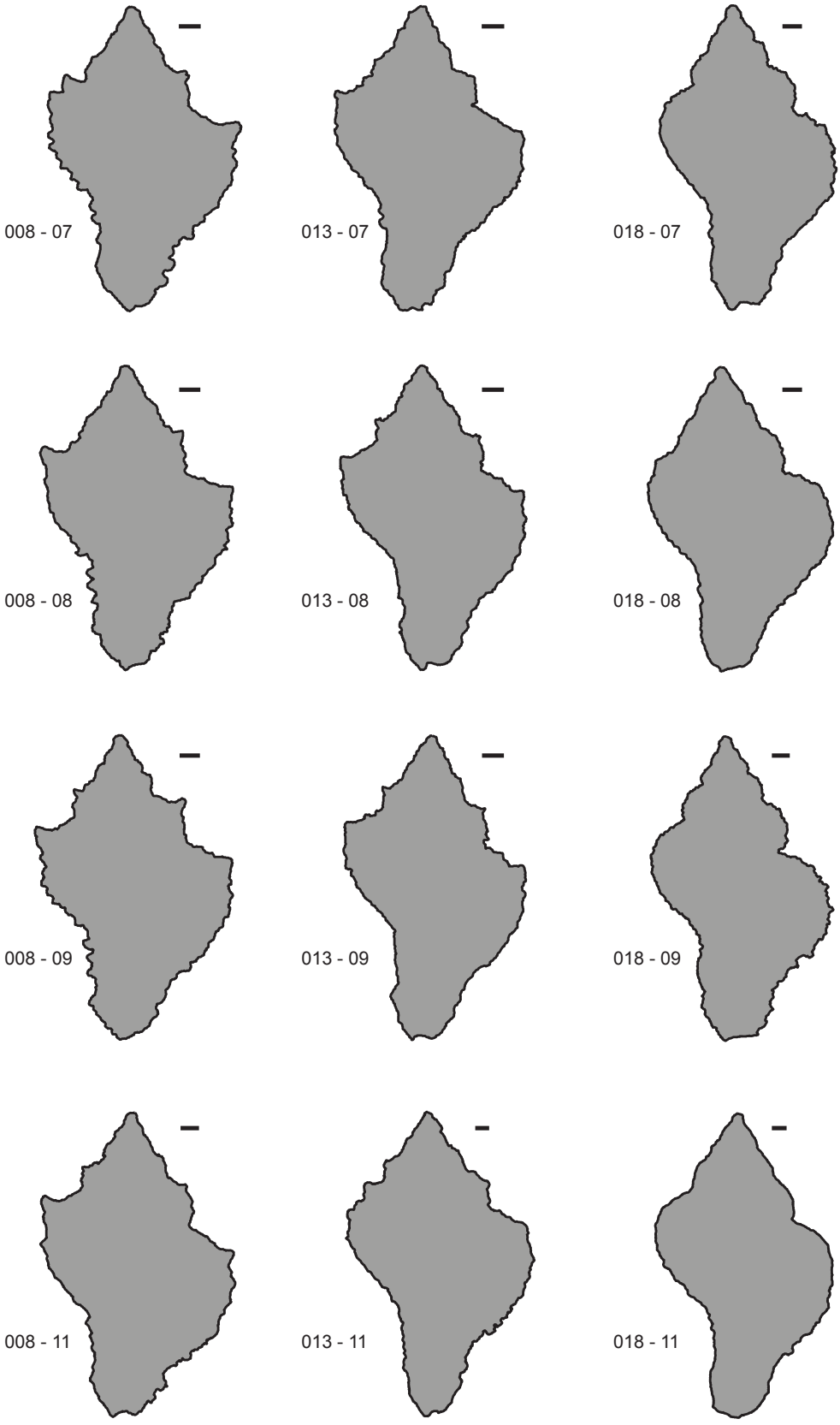


Fig. 17: Shell contours of three variants, raised in the same tank (*tank 1*) over time. These variants represent the extent of shape variation found in our sample. **First column**: snail 008. **Second column**: snail 013. **Third column**: snail 018. Note the change in apertural shape over time, and the maximal development of the spine in intermediate stages of ontogeny (Snail 008.05-07).


008 - 07
013 - 07
018 - 07
008 - 08
013 - 08
018 - 08
008 - 09
013 - 09
018 - 09
008 - 11
013 - 11
018 - 11

Also, the higher the *MGHT*, the higher the GR_{min} in the quiescent phase (rho = 0.32, $p < 0.01$, Figs. 18c, e).

From the age of 100 to 400 *DAH*, the mean number of growth halts per month (*MGHT*) is about 2 for the snails exhibiting more widely spaced growth halts (*MGHS* superior to 29 degrees). The *MGHT* is somewhat higher (about 2.3) for the snails which exhibit more closely spaced growth halts (*MGHS* inferior to 26 degrees). But the latter snails generally start at a higher number of growth halts per month and this number rapidly decreases (4-5 to 1) whereas the former have a less variable number of growth halts per month (2 to 0).

The *MGHT* is correlated with the size at 100 *DAH* (rho = -0.43, p = 0) and with the mean size over 100-400 *DAH* (rho = -0.31, $p < 0.01$), smaller snails tending to have a higher *MGHT*. However, the residuals of *MGHT* against these size measures are still correlated with intrinsic growth rates and the mean spacing between growth halts (*MGHS*) (rho = -0.31, $p < 0.01$ and rho = -0.25, $p < 0.01$, respectively).

At the scale of growth segments, at about 200 *DAH*, it was observed that snails which frequently stopped growing (high *MGHT*) generally did so for a few days whereas those that rarely stopped growing (low *MGHT*) did so for a longer time (two weeks or more). In particular, the latter snails are those for which a longer quiescent phase (no growth) is observed on the growth curves. It has also been observed that the snails which exhibited longer quiescent phases and more widely spaced growth halts tended to be visibly thicker. Although shell thickness has not been quantified, it indicates that shell secretion would remain uninterrupted during the quiescent phases.

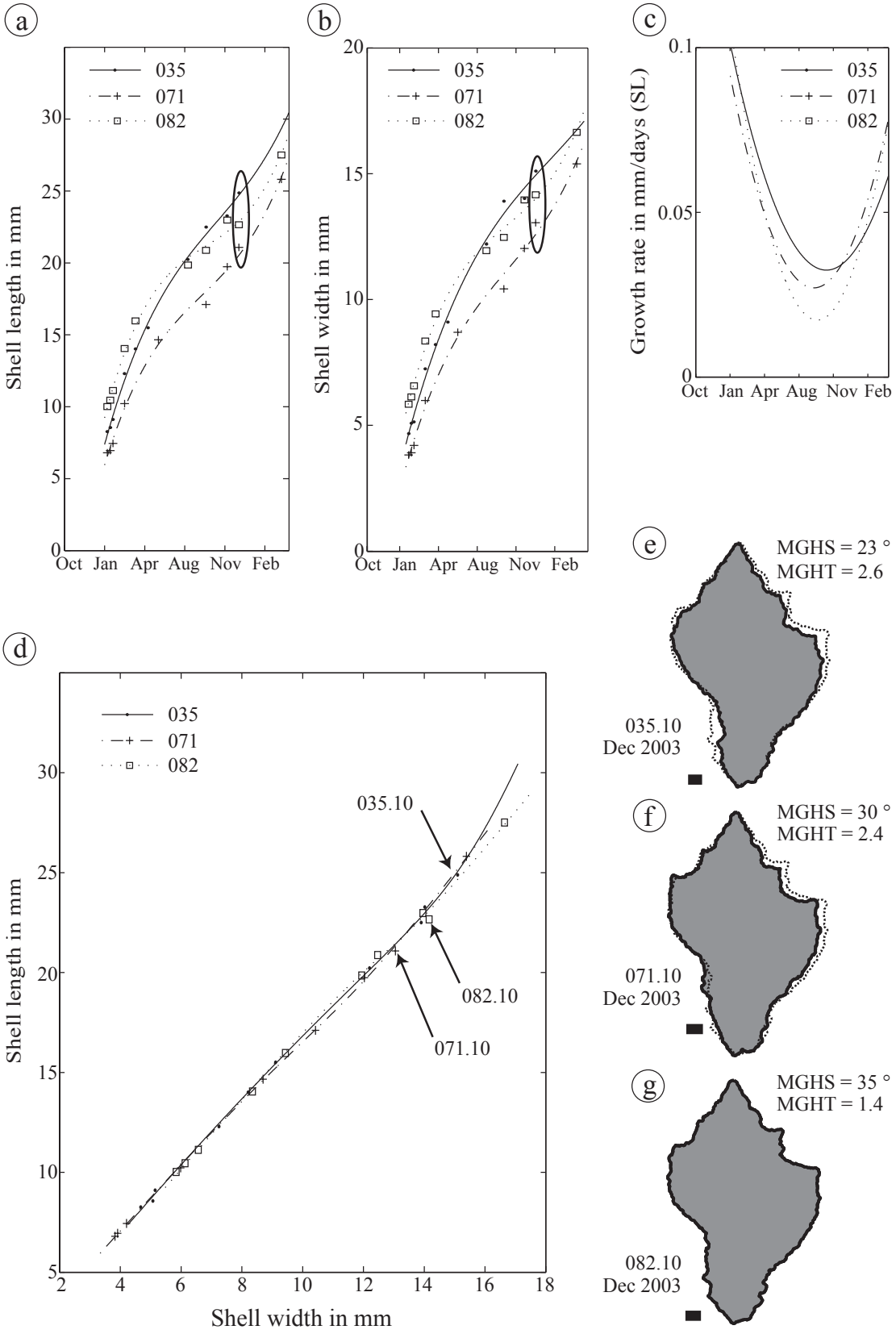
Since nearly all factors accounting for aperture shape variation in the dataset are more or less strongly correlated (Fig. 19, see Table 5 in appendices) with the mean number of growth halts per month (*MGHT*) and the mean spacing between growth halts (*MGHS*), some of the variation in shell shape seems to be accounted for by variation in growth rhythm (*MGHT*) and size of shell segments (*MGHS*). Similarly, the growth rhythm (continuous/discontinuous growth = high/low *MGHT*) seems to be related to the global shape of the growth curves (Figs. 18-19).

IV. Discussion

(1) Origin of variation

This study reveals extensive variation in growth rates, growth rhythm and shell shape. As reported above, the snails with the more 'linear' growth curves tend to have a larger size at t_0 (l_2). These snails are mainly to be found in *tanks 2 & 3*. Interestingly, these tanks are represented by a high proportion of small *IDs* (median <60). They were also the first tanks to be filled with the snails hatched before mid-October 2002. Although the correlation between l_2 and l_3 could have some biological validity, it does not seem that their estimated larger size at t_0 would result primarily from the underestimation of the real duration between the date of hatching and the date of first measurements. Corrections for the date of hatching failed to provide a reasonably good fit for both measured size at hatching and the remaining data for all snails. In fact, allowing

Fig.18: Growth curves and allometry in three variants of similar size in *tank 1*. **a**: *SL* versus time. **b**: *SW* versus time. **c**: growth rate (in mm *SL* / day) versus time. **d**: traditional biometrics and allometry. Note that the curves are gently curved. **e**: shell shape in December 2003. The shell contour of snail 082 is redrawn (dashed line) over the shell contours of snails 035 and 071. Note that the lower GR_{min} (**c**, snail 082), the larger the MGHS, the wider the aperture and the spinier the shell.


a
b
c
035
071
082
Shell length in mm
Shell width in mm
Growth rate in mm/days (SL)
Oct Jan Apr Aug Nov Feb
d
035.10
082.10
071.10
Shell length in mm
Shell width in mm
e
MGHS = 23 °
MGHT = 2.6
035.10
Dec 2003
f
MGHS = 30 °
MGHT = 2.4
071.10
Dec 2003
g
MGHS = 35 °
MGHT = 1.4
082.10
Dec 2003

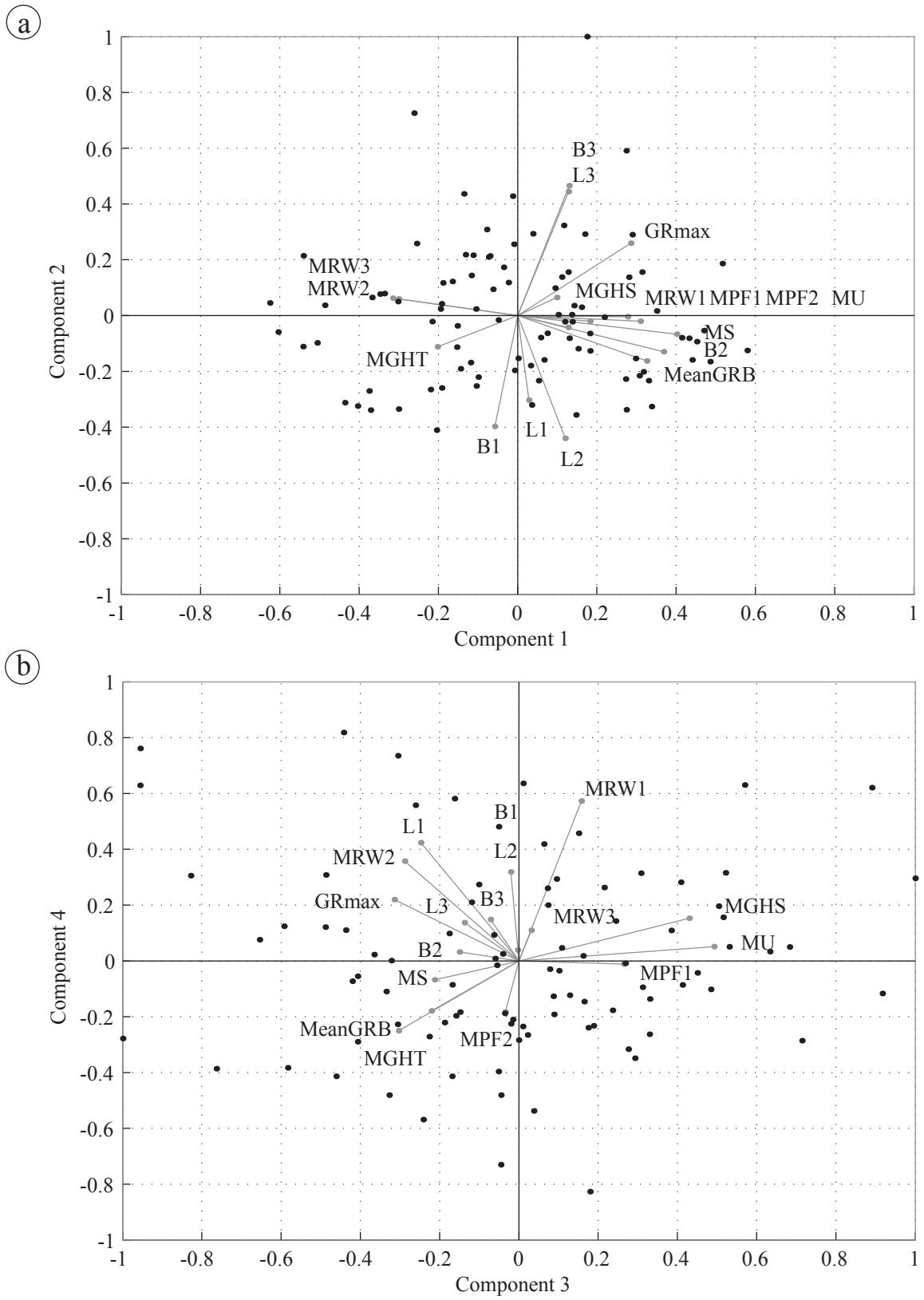


Fig. 19: Principal factor analysis of estimated growth parameters and shape variables. **a**: component 2 *vs*. component 1. **b**: component 4 *vs*. component 3. The dots represent the principal scores for each snail (92). The direction and length of blue vectors indicate how each variable contributes to the two principal components in the plot. Parallel vectors indicate that the variables are highly correlated (see also Tables 4-5 in appendices).

the onset of growth curves to shift between ± 20 days about t_0 did not improved the fittings for the snails whose size at t_0 was already over-estimated. Also, these corrections did not significantly affect the temporal pattern of variation in this population.

Then, most of the variation in size, shape and growth across and among tanks seems related to the variability in the time duration between hatching and capture. Also, it suggests that the shape of the growth curves (b_3, l_3) could have something to do with the time spent as free living juveniles in the hatching tank. The snails with more 'linear' growth curves (low b_3, low l_3) are mainly those that were let longer free in the hatching tank. During the first three weeks after the beginning of hatching, the snails did not have an easy access to food sources in the hatching tank. This resulted in an important intra-specific predation. Also, once they were caught, the first hatchlings were fed twice a week (on Mondays and Thursdays) for a couple of weeks (until the beginning of November). It may have influenced growth and growth rhythm, since only these snails' growth cannot be reasonably fitted by a logistic growth curve (slope break).

Moreover, the growth curves parameters seem quite dependent on the tanks. For example, even for snails which were captured soon after hatching at the end of October 2002, fed weekly from the beginning and bred in *tanks 2 & 3*, we observe that they tend to have a flatter growth curve than those bred in *tanks 1 & 4*. This could reflect some tank effect. If so, the source of this variation remains unclear.

The quiescent phase could also be related to environmental effects, since it happens nearly synchronously among tanks during summer 2003. This could be due to a small increase of temperature because of a heat wave, particularly strong in summer 2003. Although the air temperature of the room was thermostatically regulated, a slight increase of this temperature (up to 3 °C) was observed during these months. Thus, the quiescent phase could represent a seasonal pattern (though the variation in water temperature is not comparable to what found in nature), but a longer term experiment would be necessary to test this hypothesis. Also, it would remain unclear why a seasonal slow down of growth rates did not affect equally all snails[22]. We rather suggest that variation in 'growth curve types' results from the variation in shell growth rhythm (*MGHT*). In our view, the quiescent phase is identified in 80% of the snails in the sample at about 285-350 *DAH* because the time spent on a growth halt becomes sufficiently long in most snails of this age for our monthly measurements to reveal it.

(2) Dynamic pattern of variation

Given the non linearity of ontogenetic trajectories, the extent of variation is obviously time-dependent. For instance, the lastly hatched snails (*tank 6*) are significantly smaller at 100 *DAH* than those in the other tanks, but at 500 *DAH*, the size differences tend to vanish (Figs. 6-9). It comes from the fact that the first hatched snails had either a slow to moderate but relatively constant growth rate (mainly *tanks 2 & 3*), or a high to moderate growth rate followed by a relatively long quiescent phase (mainly *tanks 1 & 4*). The last hatched snails (*tank 6*) spent less time as free living juveniles and they tend to have an

22 Vasconcelos *et al.*, unpublished data, obtained a mean annual growth curve roughly similar to our individual growth curves (snails raised in laboratory, under natural sea water, hatched the same day, see Vasconcelos *et al*., 2004 for a description of their experiment). However, this curve highlights a slow down of growth rates in winter. Interestingly, the snails studied by Vasconcelos *et al.* (unpublished) hatched in spring. It means that their quiescent phase is situated at about 6 months after hatching. In our sample, this quiescent phase is located about 10-11 months after hatching (in summer).

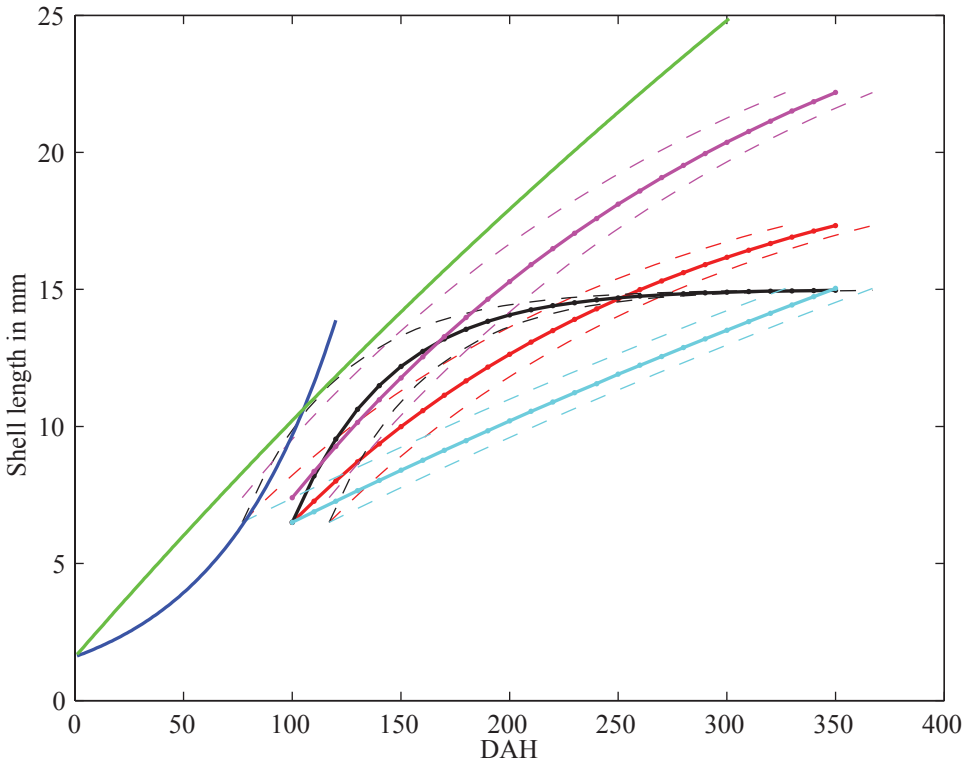


Fig. 20: Comparison of our individual growth curves (red, magenta, cyan, black) with that proposed by Vasconcelos *et al.* (2004) (blue) and Vasconcelos *et al.* (2006) (green) for *Hexaplex trunculus*. The broken lines represent the uncertainty on the date of hatching for each individual growth curve.

intermediate growth rate and a relatively early and short quiescent phase. Consequently, over the duration of the experiment, the mean growth rates are not different among tank replicates or among 'growth curves types' although they can be different on smaller (or larger) time scales.

(3) Comparison with other studies

Some growth curves have been proposed by Vasconcelos *et al*. (2004) for the first four months after hatching (Fig. 20, blue) of snails raised in laboratory and by Vasconcelos *et al*. (2006) for mark-recapture experiments of snails in the field (Fig. 20, green). Given the uncertainty of the date of hatching, our individual growth curves provide similar estimations (Fig. 20, red, magenta, cyan, black), though they emphasize an extensive variation.

(4) Growth vector model: origin of covariation among shell characters

The PCA on growth and shape parameters tends to point out that some of the variation in growth halts spacing (*MGHS*), aperture shape (allometry, ornamentation) and variation in growth curve shape (b_3, l_3) could be related to variation in growth rhythm (*MGHT*) (Fig. 19).

A simple model can reproduce some of the effects of growth rhythm on shell shape highlighted in this study. As a first simplification, we assume that the time elapsed between successive growth lines is constant over ontogeny and among specimens (see chapters 3 & 4, null hypothesis 1). We assume that all specimens start with the same initial growth vector map which is scaled isometrically over ontogeny (null hypothesis 2). However, we assume that growth tends to be

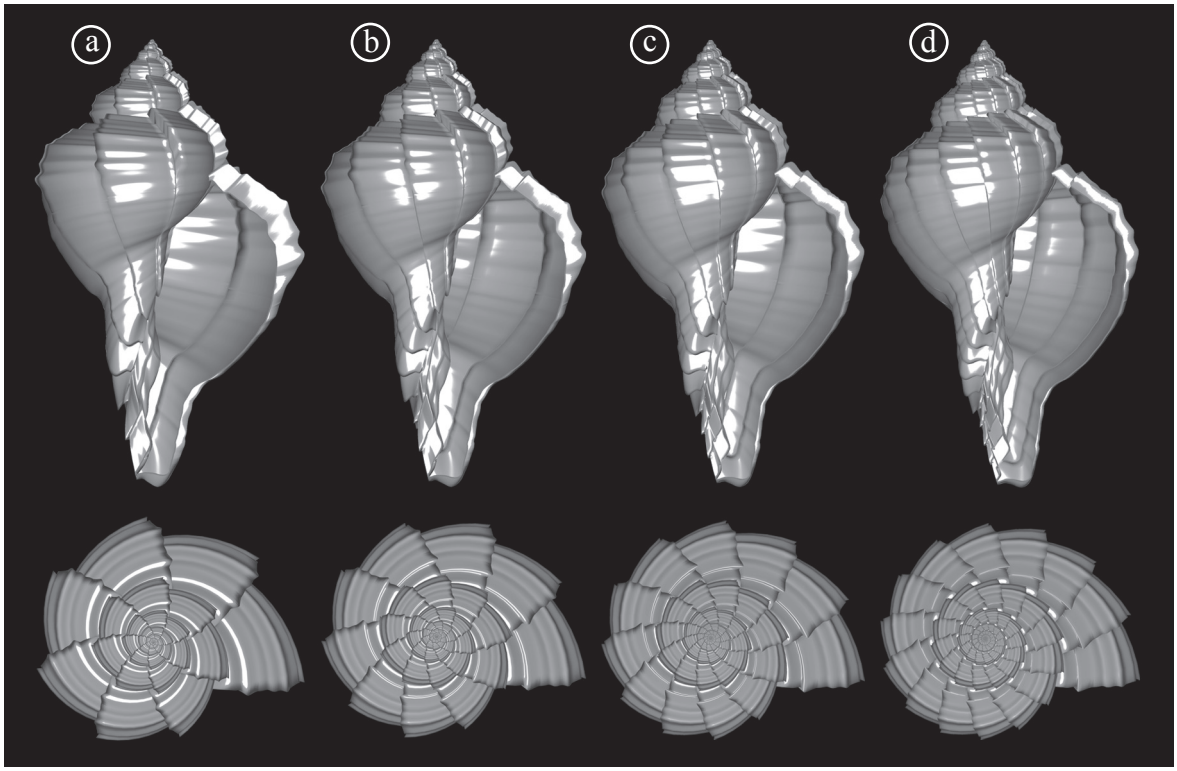


Fig. 21: Simulations of *Hexaplex*-like shells, using the same growth vector maps. Consecutive apertures change their shape locally, by growing larger spines. **a-d :** by construction (null hypothesis 4), successive growth halts occur respectively every 8, 6, 5, and 4 growth increments. Note that from left to right, the increase in the number of growth halts per whorl results in smoother and slender shells.

faster in some spatial locations corresponding to spiral chords, especially in the posterior part of the free aperture margin (spatially differentiated growth vector map, new rule). Then (this is actually the goal of this new rule), the spiral chords tend to develop into 'spines' over ontogeny. These conditions result in shells which rapidly exhibit unreasonably strong spines. The aperture also tends to become relatively wider in the course of ontogeny. This is the "no growth halts case" which is not different among specimens.

We simulate the process of growth halts formation phenomenologically. We assume that when growth stops, aperture outline becomes smoother and a smaller, in order to simulate the mantle deflation observed after the formation of a growth halt. This 'smoothing' is simulated by warping the current aperture to a smoother one. For simplicity, this smoother aperture is not changed during ontogeny nor among specimens (null hypothesis 3). Hence, after the formation of a growth halt, aperture shape is similar among specimens. Then, growth resumes by applying the same growth vector map, starting from this smoother, smaller aperture.

We assume that the number of growth halts per time unit remains constant over ontogeny (null hypothesis 4) but this number is allowed to vary among specimens. In the simulations above, a growth halt is built every 4 to 8 increments (see Figs. 21d-a). This results in more and more closely spaced growth halts from left to right (about 8, 10, 12 and 14 growth halts per whorl, Figs. 21a-d).

This model highlights that variation in number of growth halts per time unit (and growth halts spacing) results in introducing some variation in the intensity of ornamentation (Fig. 21).

More widely spaced growth halts are associated with stronger ornamentation, simply because the number of increments between successive growth halts is larger. There is no 'need' to introduce variation in the growth of these increments among specimens (and over ontogeny) to obtain this effect. Note also, that for the same reason, the specimens with the more widely spaced growth halts tend to exhibit a relatively wider aperture (more globose shell). Then, the growth vector model points out that the size of ornamental features and degree of aperture allometry are expected to scale up with the spacing between growth halts.

V. Conclusions

Although all snails originated from a single egg mass, the time of hatching was quite variable. Some of the variation in size, growth and shape among tanks seems to result from asynchrony in hatching time and the variability in the time elapsed between hatching and capture. The fact that the first hatchlings spent the longest duration as free living juveniles and that they have been fed twice a week for a couple of weeks might have influenced their growth dynamics: flattened growth curves (100-560 *DAH*), relatively closely spaced growth halts, low allometry of aperture shape, moderate ornamentation. Living conditions could also be responsible for some of the tank effects.

The quiescent phase, as put in evidence for 80 percent of the snails, could be due to a small increase of the temperature during that summer, but a longer term experiment. would be required for testing this hypothesis. If so, it is not clear why a seasonal slow down of growth rates did not affect all snails.

At the scale of growth segments, it was observed that snails which frequently stopped growing (high *MGHT*) generally did so for a few days (3 to 5 at about 200 *DAH*) whereas those that rarely stopped growing (low *MGHT*) did so for a longer time (two weeks at about 200 *DAH*). In particular, the latter snails are those for which a longer quiescent phase (no growth) was observed on the growth curves (up to two months at about 285-350 *DAH*). Thus, the time spent on a growth halt seems to increase exponentially with age and variation in growth rhythm is extensive. Although our monthly measurements do not allow us to quantify this precisely, the growth rhythm (frequency and amplitude of pulses of growth; continuous/discontinuous growth = high/low *MGHT*) is related to the global shape of the growth curves, the mean spacing between growth halts and the differences in mean shell shape among snails.

This study highlights how variation in shell growth rhythm can impinge on the spacing between growth halts, the general shape of growth curves and the allometry and ornamentation of the aperture. A simple model can reproduce some of the effects of growth rhythm on shell shape as stressed by this study. The growth vector model points out that the size of ornamental features and degree of aperture allometry are expected to scale up with the spacing between growth halts. Variation in growth rhythm (number of growth halts per specified unit time) is then viewed as critical for generating the observed covariation between growth halts spacing and intensity of ornamentation. The covariation between these shells characters seems, at least at first glance, similar to that observed on highly variable ammonoids species.

Although growth halts are often regarded as adaptations to particular modes of life, we suggest that ammonoids and gastropods share

similar growth rules, tied to basic constraints of accretionary growth. We reject the hypothesis that the growth segments between two successive growth halts represent an equal duration over ontogeny and among specimens. The spacing between growth halts is also shown not to be as regular as often thought, putting doubts on their adaptive significance. If growth halts are to be attributed an adaptive function (e.g. Spight & Lyons, 1974; Savazzi & Sasaki, 2004), authors should recognize that it would be restricted to particular intermediate ages. In early development, the time spent on a growth halt could be too short to provide any advantage and in older shells, growth halts may be completely abraded.

VI. References

Bandel, K. (1975). Das Embryonalgehäuse mariner Prosobranchier der Region von Banyuls-sur-Mer. *Vie MilieuSérie A- Biologie Marine* **25,** 83-118.

Bayer, U. (1970). Anomalien bei Ammoniten des Aaleniums und Bajociums und ihre Beziehung zur Lebensweise. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen* **135,** 19-41.

Bond, P. N. & Saunders, W. B. (1989). Sublethal injury and shell repair in upper Mississippian ammonoids. *Paleobiology* **15,** 414-428.

Bookstein, F. L. (1989). Principal warps: thin-plate splines and the decomposition of deformations. *IEEE Transactions on Pattern Analysis and Machine Intelligence* **11,** 567-585.

Bookstein, F. L. (1991). *Morphometric Tools for Landmark Data*. Cambridge University Press, Cambridge.

Bookstein, F. L. (1996). Biometrics, biomathematics and the morphometric synthesis. *Bulletin of Mathematical Biology* **58,** 313-365.

Bucher, H. (1997). Caractères périodiques et mode de croissance des ammonites: comparaison avec les gastéropodes. *Geobios Mémoire Spécial* **20,** 85-99.

Bucher, H. & Guex, J. (1990). Rythmes de croissance chez les ammonites triasiques. *Bulletin des Laboratoires de Géologie, Minéralogie, Géophysique et du Musée géologique de l'Université de Lausanne* **308,** 191-209.

Bucher, H., Landman, N. H., Klofak, S. M. & Guex, J. (1996). Mode and rate of growth in ammonoids. In *Ammonoid paleobiology* (ed. N. H. Landman), pp. 408-461. Plenum Press, New-York.

Burnaby, T. P. (1966). Allometric growth of ammonoid shells: a generalization of logarithmic spiral. *Nature* **209,** 904-906.

Checa, A., Company, M., Sandoval, J. & Weitschat, W. (1996). Covariation of morphological characters in the Triassic ammonoid *Czekanowskites rieberi*. *Lethaia* **29,** 225-235.

Checa, A. G. & Jimenez-Jimenez, A. P. (1997). Regulation of spiral growth in planorbid gastropods. *Lethaia* **30,** 257-269.

Checa, A. G., Jimenez-Jimenez, A. P. & Rivas, P. (1998). Regulation of spiral coiling in the terrestrial gastropod *Sphincterochila*: an experimental test of the road-holding model. *Journal of Morphology* **235,** 249-257.

Checa, A. G., Okamoto, T. & Keupp, H. (2002). Abnormalities as natural experiments: a morphogenetic model for coiling regulation in planispiral ammonites. *Paleobiology* **28,** 127-138.

Chirat, R., Enay, R., Hantzpergue, P. & Mangold, C. (2008). Developmental integration related to buoyancy control in Nautiloids: evidence from unsusual septal approximation and ontogenetic allometries in a Jurassic species. *Palaeontology* **51,** 251-261.

D'Asaro, C. N. (1988). Micromorphology of Neogastropod egg capsules. *Nautilus* **102,** 134-148.

D'asaro, C. N. (1991). Gunnar Thorson's world-wide collection of prosobranch egg capsules: Muricidae. *Ophelia* **35,** 1-101.

Dagys, A., Bucher, H. & Weitschat, W. (1999). Intraspecific variation of *Parasibirites kolymensis* Bychkov (Ammonoidea) from the Lower Triassic (Spathian) of Arctic Asia Mitteilungen. *Geologisches-Paläontologisches Institut Universität Hamburg* **83,** 163-178.

Dagys, A. S. & Weitschat, W. (1993). Extensive intraspecific variation in a Triassic ammonoid from Siberia. *Lethaia* **26,** 113-121.

Ferson, S., Rohlf, F. J. & Koehn, R. (1985). Measuring shape variation of two-dimensional outlines. *Systematic Zoology* **34,** 59-68.

Fretter, V. & Graham, A. (1994). *British Prosobranch Molluscs. Their functional anatomy and ecology*. The Ray Society, London.

Guex, J. (1967). Contribution à l'étude des blessures chez les ammonites. *Bulletin géologique de Lausanne* **165,** 1-23.

Guex, J. (1968). Sur deux conséquences particulières des traumatismes du manteau des ammonites. *Bulletin*

de la Societé vaudoise des sciences naturelles.

Guex, J., Koch, A., O'Dogherty, L. & Bucher, H. (2003). A morphogenetic explanation of Buckman's law of covariation. *Bulletin de la Société géologique de France* **174,** 603-606.

Hammer, O. & Bucher, H. (1999). Reaction-diffusion processes: application to the morphogenesis of ammonoid ornamentation. *Geobios* **32,** 841-852.

Hammer, O. & Bucher, H. (2005a). Buckman›s first law of covariation: a case of proportionality. *Lethaia* **38,** 67-72.

Hammer, O. & Bucher, H. (2005b). Models for the morphogenesis of the molluscan shell. *Lethaia* **38,** 111-122.

Houart, G. (2001). *A Review of the Recent Mediterranean and Northeastern Atlantic Species of Muricidae*. Evolver SRL, Rome.

Hutchinson, J. M. C. (1989). Control of gastropod shell shape: the role of the preceding whorl. *Journal of Theoretical Biology* **140,** 431-444.

Illert, C. (1981). The growth and feeding habits of a South Australian Murex. *Of Sea and Shore* **12,** 9-10.

Illert, C. (1990). *Nipponites mirabilis*: a challenge to seashell theory. *Nuovo Cimento della Societa Italiana di Fisica D - Condensed Matter Atomic Molecular and Chemical Physics Fluids Plasmas Biophysics* **12,** 1405-1421.

Kniprath, E. (1981). Ontogeny of the molluscan shell field: a review. *Zoologica Scripta* **10,** 61-79.

Knudsen, J. (1950). Egg capsules and development of some marine prosobranchs from tropical West Africa. *Atlantid Reports* **1,** 85-130.

Kuhl, F. P. & Giardina, C. R. (1982). Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* **18,** 236-258.

Landman, N. H. & Waage, K. M. (1986). Shell abnormalities in scaphitid ammonites. *Lethaia* **19,** 211-224.

Linsley, R. M. (1977). Some "laws" of gastropod shell form. *Paleobiology* **3,** 196-206.

Linsley, R. M. (1978). Shell form and the evolution of gastropods. *American Scientist* **66,** 432-441.

Martín, P., Sánchez, P. & Ramón, M. (1995). Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research* **23,** 319-331.

Monnet, C. & Bucher, H. (2005). New middle and late Anisian (Middle Triassic) ammonoid faunas from northwestern Nevada (USA): taxonomy and biochronology. *Fossils and Strata* **52,** 1-60.

Morita, R. (1991a). Finite element analysis of a double membrane tube (DMS-tube) and its implication for gastropod shell morphology. *Journal of Morphology* **207,** 81-92.

Morita, R. (1991b). Mechanical constraints on aperture form in gastropods. *Journal of Morphology* **207,** 93-102.

Morita, R. (2003). Why do univalve shells of gastropods coil so tightly? A head-foot guidance model of shell growth and its implication on developmental constraints. In *Morphogenesis and Pattern Formation in Biological Systems: Experiments and Models* (ed. T. Sekimura, S. Noji, N. Ueno and P. K. Maini), pp. 345-354. Springer-Verlag, Tokyo.

Muzavor, S. & Morenito, P. M. (1999). Roteiro Ecológico da Ria Farmosa. In *Molusco Gastrópodos*, vol. IV, Universidade do Algarve, Faro.

Okamoto, T. (1988). Analysis of heteromorph ammonoids by differential geometry. *Palaeontology* **31,** 35-52.

Ramón, M. & Amor, M. J. (2001). Increasing imposex in populations of *Bolinus brandaris* (Gastropoda: Muricidae) in the northwestern Mediterranean. *Marine Environmental Research* **52,** 463-475.

Ramón, M. & Amor, M. J. (2002). Reproductive cycle of *Bolinus brandaris* and penis and genital duct size variations in a population affected by imposex. *Journal of the Marine Biological Association of the United Kingdom* **82,** 435-442.

Raup, D. M. (1966). Geometric analysis of shell coiling: some general problems. *Journal of Paleontology* **40,** 1178-1190.

Rice, S. H. (1998). The bio-geometry of mollusc shells. *Paleobiology* **24,** 133-149.

Rohlf, F. J. (1998). On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Systematic Biology* **47,** 147-158.

Rohlf, F. J. & Bookstein, F. L. (2003). Computing the uniform component of shape variation. *Systematic Biology* **52,** 66-69.

Rohlf, F. J. & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39,** 40-59.

Romero, M. S., Gallardo, C. S. & Bellolio, G. (2004). Egg laying and embryonic-larval development in the snail *Thais (Stramonita) chocolata* (Duclos, 1832) with observations on its evolutionary relationships within the Muricidae. *Marine Biology* **145,** 681-692.

Savazzi, E. (1990). Biological aspects of theoretical shell morphology. *Lethaia* **23,** 195-212.

Savazzi, E. & Sasaki, T. (2004). Synchronized sculpture in gastropod shells. *American Malacological Bulletin* **18,** 87-114.

Schmittbuhl, M., Allenbach, B., Le Minor, J.-M. & Schaaf, A. (2003). Elliptical descriptors: some simplified morphometric parameters for the quantification of complex outlines. *Mathematical Geology* **35,** 853-871.

Simkiss, K. & Wilbur, K. M. (1989). *Biomineralization: Cell Biology and Mineral Deposition*. Academic Press, San Diego.

Smith, B. S. (1971). Sexuality in the American mud snail *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London* **39,** 377-378.

Spight, T. M. & Lyons, A. (1974). Development and functions of the shell sculpture of the marine snail *Ceratostoma foliatum*. *Marine Biology* **24,** 77-83.

Thompson D'Arcy, W. (1952). *On Growth and Form*, Second edition. Cambridge University Press, Cambridge.

Vasconcelos, P., Gaspar, M. B. & Castro, M. (2006). Imposex in *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast - southern Portugal). *Marine Pollution Bulletin* **52,** 337-341.

Vasconcelos, P., Gaspar, M. B., Joaquim, S., Matias, D. & Castro, M. (2004). Spawning of *Hexaplex (Trunculariopsis) trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryonic development, hatchling and juvenile growth rates. *Invertebrate Reproduction & Development* **46,** 125-138.

Vasconcelos, P., Gaspar, M. B., Pereira, A. M. & Castro, M. (2006). Growth rate estimation of *Hexaplex (Truncularioposis) trunculus* (Gastropoda: Muricidae) based on mark/recapture experiments in the Ria Formosa lagoon (Algarve coast, southern Portugal). *Journal of Shellfish Research* **25,** 249-256.

Verhulst, P. F. (1838). Notice sur la loi que la population suit dans son accroissement. *Correspondance Mathématique et Physique* **10,** 113-121.

Von Bertalanffy, L. (1938). A quantitative theory of organic growth: inquiries on growth laws. *Human Biology* **10,** 181-213.

Westermann, G. E. G. (1966). Covariation and taxonomy of the Jurassic ammonite *Sonninia adicra* (Waagen). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* **124,** 289-312.

VII. Appendices

Table 1: One way analysis of variance of growth parameters					
Kruskal-Wallis ANOVA Table			**Tmax**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	6772.8002	4	1693.2	9.499	0.049767
Error	58110.1998	87	667.9333		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**GRmax**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	9736.1729	4	2434.0432	13.6552	0.008481
Error	55146.8271	87	633.8716		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**GRmeanB**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	9002.5508	4	2250.6377	12.6263	0.013254
Error	55880.4492	87	642.304		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**GRmeanL**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	9240.3796	4	2310.0949	12.9599	0.011474
Error	55642.6204	87	639.5703		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**B3**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	17804.0968	4	4451.0242	24.9707	5.10E-05
Error	47078.9032	87	541.1368		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**L3**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	15876.4841	4	3969.121	22.2672	1.77E-04
Error	49006.5159	87	563.2933		
Total	64883	91			
Kruskal-Wallis ANOVA Table			**Tmin**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	3006.8103	4	751.7026	8.6735	0.069799
Error	18833.1897	59	319.2066		
Total	21840	63			
Kruskal-Wallis ANOVA Table			**GRmin**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	5040.9673	4	1260.2418	14.5413	0.005754
Error	16799.0327	59	284.7294		
Total	21840	63			

Table 2: One way analysis of variance of size					
Kruskal-Wallis ANOVA Table			**Size at 0 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	10324.6667	4	2581.1667	21.7361	0.000226
Error	24825.3333	70	354.6476		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Size at 100 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	7811.3333	4	1952.8333	16.4449	0.002477
Error	27338.6667	70	390.5524		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Size at 200 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	6481.0667	4	1620.2667	13.6444	0.008521
Error	28668.9333	70	409.5562		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Size at 300 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	5713.3333	4	1428.3333	12.0281	0.017144
Error	29436.6667	70	420.5238		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Size at 400 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	6109.8667	4	1527.4667	12.8629	0.011966
Error	29040.1333	70	414.859		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Size at 500 DAH**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	1743.6	4	435.9	3.6707	0.4524
Error	33406.4	70	477.2343		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Mean Shell length (Fitted): 100-400 days**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	5902.2667	4	1475.5667	12.4258	0.01445
Error	29247.7333	70	417.8248		
Total	35150	74			
Kruskal-Wallis ANOVA Table			**Mean Centroid size: data**		
Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Tanks	8305.6794	4	2076.4198	11.6489	0.020162
Error	56577.3206	87	650.314		
Total	64883	91			

Table 3: Correlations between shape variables and size / time

	Uniform1	Uniform2	U1+U2	RW1	RW2	RW3	RW4	
Centroid size	0	2.22E-15	0	0	0	5.02E-04	0.0022	p-value
	0.3861	0.2709	0.4574	0.399	-0.4652	-0.1203	-0.1057	r
Time	4.12E-32	3.91E-25	5.74E-57	5.89E-29	1.68E-27	1.21E-08	1.07E-04	p-value
	0.3925	0.3479	0.5122	0.3732	-0.3638	0.1957	-0.1337	r

	PF1	PF2	PF3	PF4	SR=SL/SW	
Centroid size	0	0.0208	2.15E-12	1.97E-13	2.56E-103	p-value
	0.4782	0.0801	0.2408	0.2517	-0.6549	r
Time	8.01E-55	1.93E-05	0.0089	8.49E-17	6.57E-60	p-value
	0.5035	-0.1474	0.0905	0.2828	-0.5237	r

	Uniform1	Uniform2	U1+U2	RW1	RW2	RW3	RW4	SR
PF1	0	4.84E-10	0	1.22E-20	0	1.43E-11	1.55E-13	8.04E-85
	0.5252	0.2139	0.5006	0.3142	-0.623	0.2318	-0.2528	-0.6062
PF2	9.83E-04	9.14E-04	0.6972	8.87E-08	0.0066	0	0.2378	4.93E-05
	0.114	-0.1147	-0.0135	-0.1842	-0.0941	-0.6107	0.0409	-0.1401
PF3	1.87E-06	0.0019	0.4033	0.0014	0.0078	0	6.68E-18	1.35E-11
	0.1644	-0.1077	0.029	0.1103	-0.0921	-0.5148	-0.2934	-0.2314
PF4	4.59E-14	3.85E-07	4.85E-18	0.04	7.78E-13	3.50E-09	2.68E-12	1.22E-10
	0.2581	0.175	0.2946	0.0711	-0.2455	0.2031	-0.2398	-0.2205

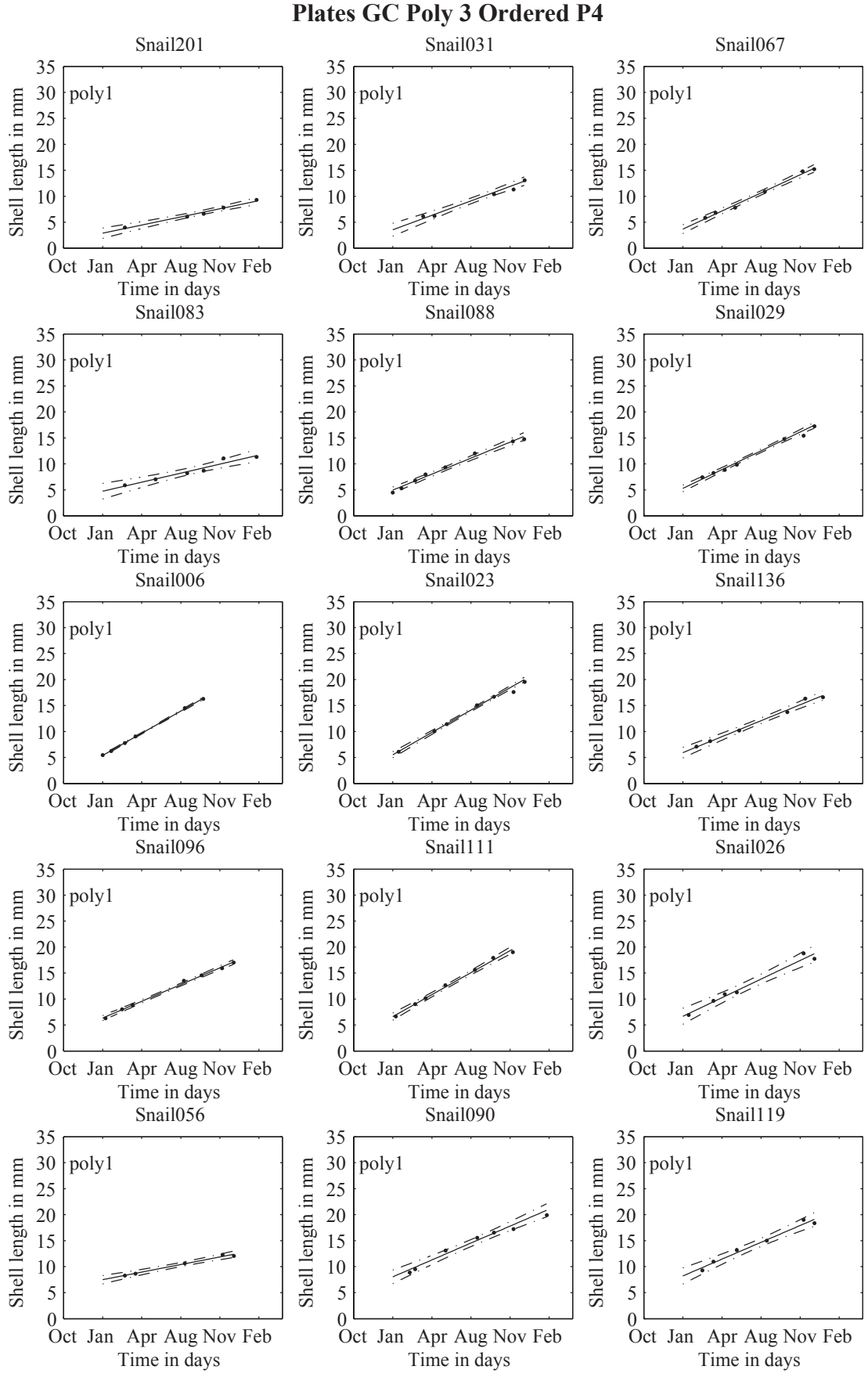
Table 4: Correlations between PCA components and original variables

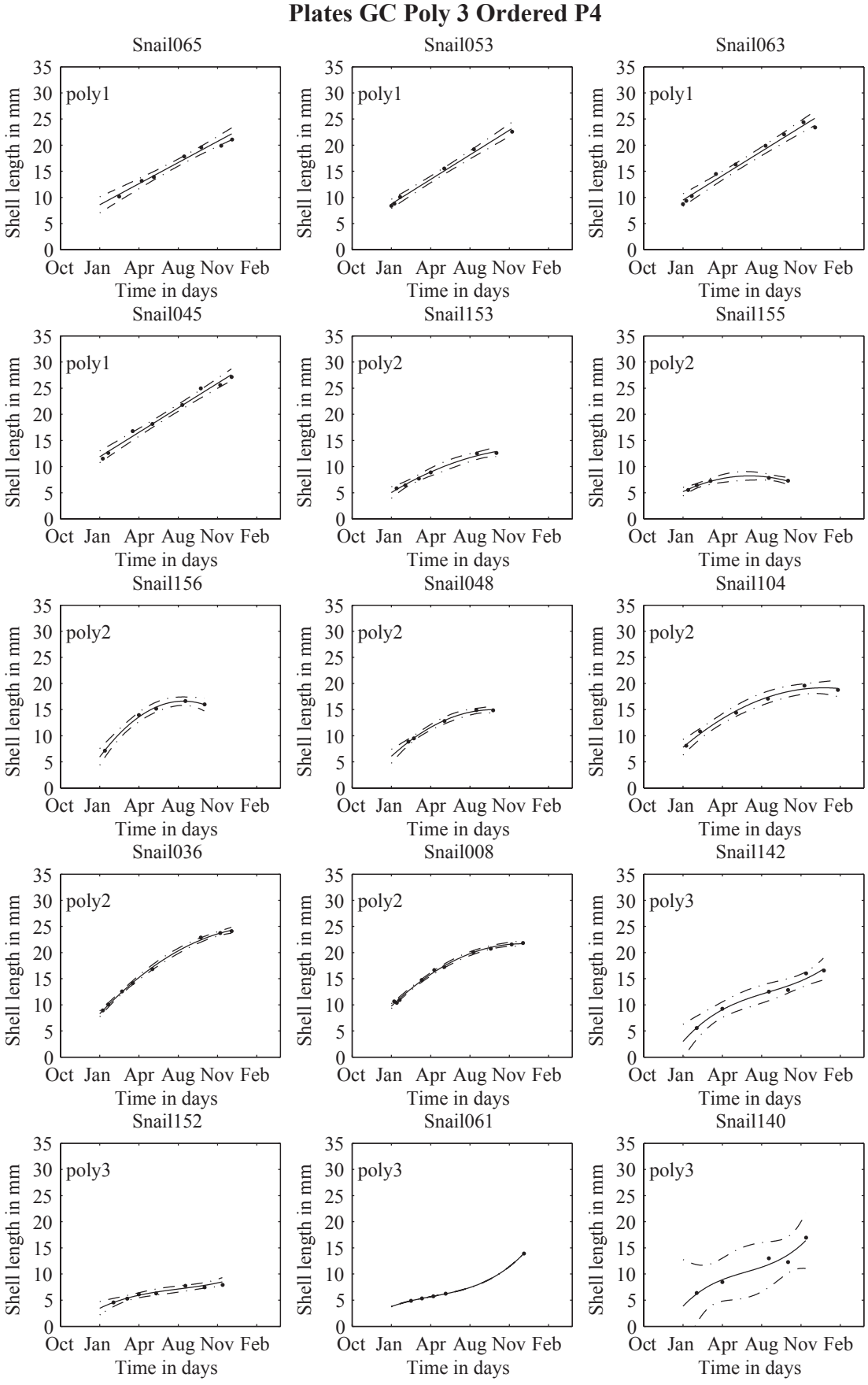
p-value	SCORE 1	SCORE 2	SCORE 3		rho	SCORE 1	SCORE 2	SCORE 3
b1	0.43802	0	0.32662		b1	-0.081716	0.92224	-0.10328
b2	0	0.008128	0.13461		b2	-0.85859	0.27511	-0.1571
b3	0.000118	0	0.23066		b3	-0.39345	-0.84208	-0.12607
l1	2.85E-06	0	0.000557		l1	-4.71E-01	0.71852	-0.35504
l2	0.004870	0	0.84941		l2	-0.29208	0.88314	-0.020036
l3	5.73E-05	0	0.1219		l3	-4.10E-01	-0.83361	-0.16237
MGHS	0.030915	0.64117	2.50E-07		MGHS	-0.22545	-0.04915	5.14E-01
MeanGRB	0	0.011731	0.000318		MeanGRB	-0.70109	0.26232	-0.36942
Grmax	0	0.000647	1.81E-05		Grmax	-0.68628	-0.35108	-4.35E-01
MGHT	5.67E-07	0.43297	0.000234		MGHT	4.94E-01	0.082742	-0.3746
MRW1	3.24E-05	0.27425	0.054378		MRW1	-4.23E-01	0.11505	0.20138
MRW2	0	0.3775	0.001252		MRW2	0.64046	-0.092952	-0.33297
MRW3	0	0.17514	0.68575		MRW3	0.66637	-0.14249	0.042677
MPF1	0	0.83466	0.001406		MPF1	-0.60595	0.022024	0.32969
MPF2	0	0.8462	0.76534		MPF2	-0.69057	0.020468	-0.031487
MU	0.012301	0.31093	9.39E-09		MU	-0.26062	0.10667	5.63E-01
MS	0	0.34417	0.003938		MS	-0.9054	0.09961	-0.29885
variance	30%	23%	10%					
explained								

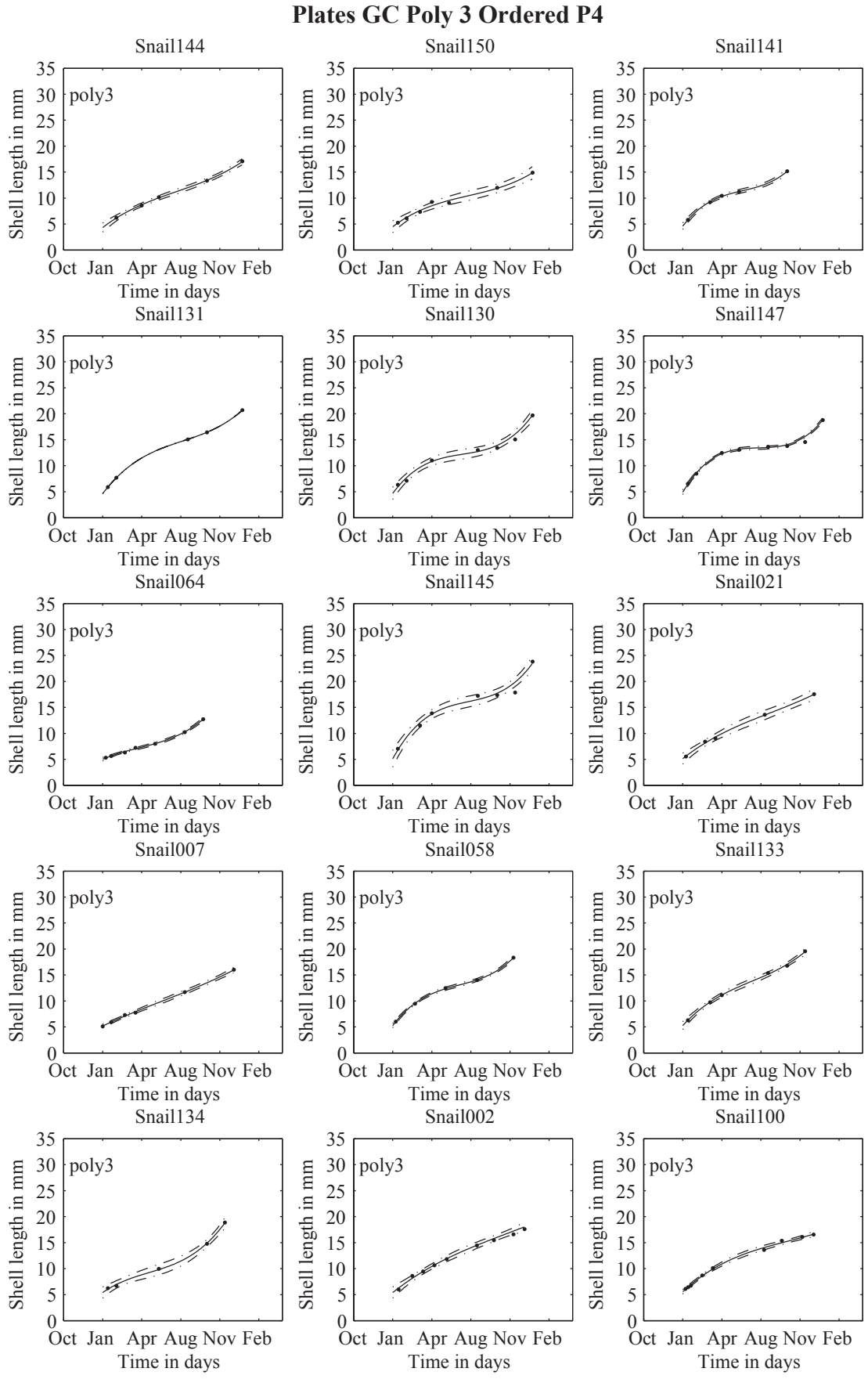
Table 5: Correlations between variables: p-values (left) and correlation coeffcient rho (right). Significant correlations (p-values < 0.05) are highlighted.

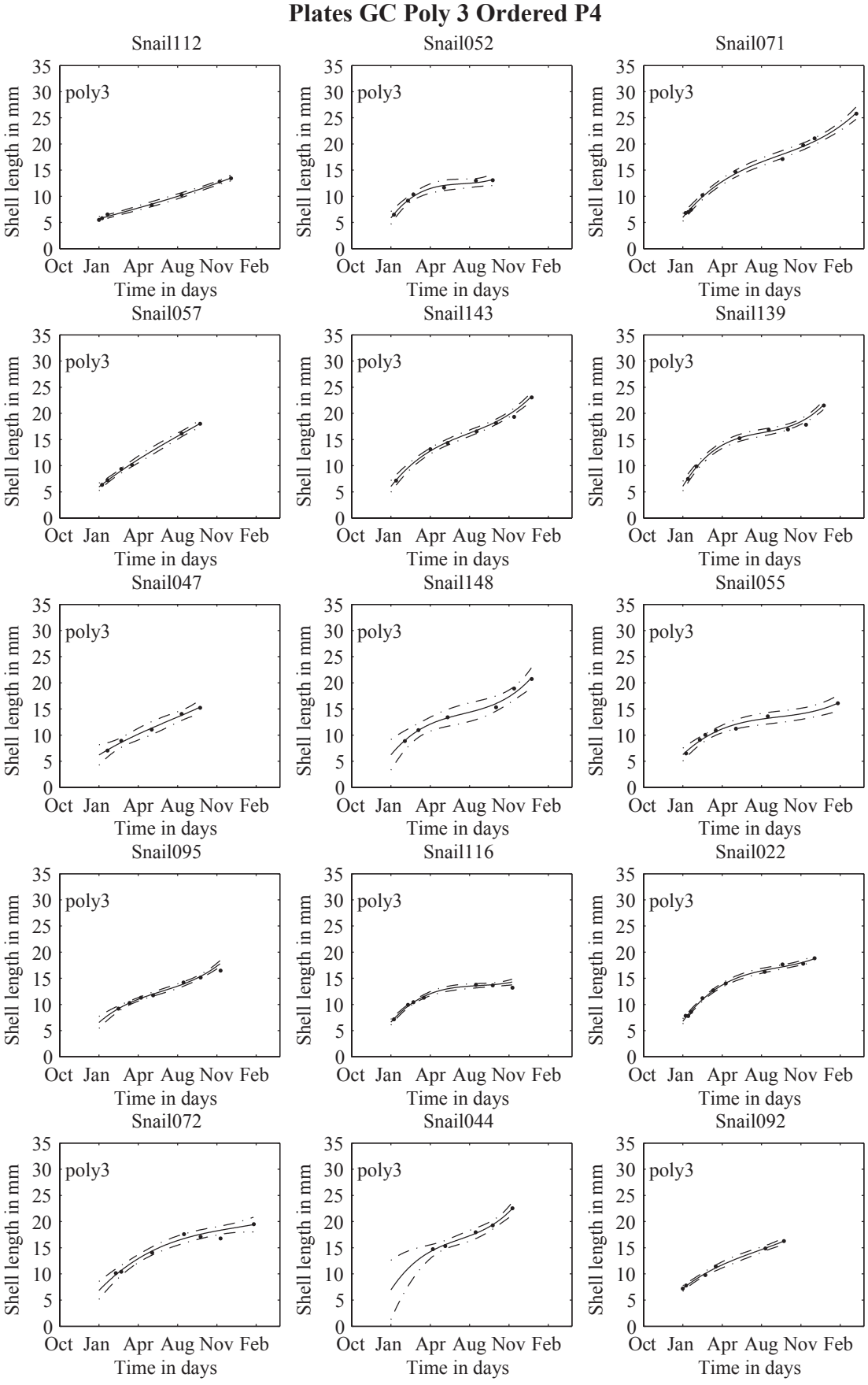
p-value	b1	b2	b3	l1	l2	l3	MGHS	MeanGRB	Grmax	MGHT	MRW1	MRW2	MRW3	MPF1	MPF2	MU	MS
b1	/	0.01	0.00	0.00	0.00	0.00	0.65	0.00	0.04	0.70	0.26	0.39	0.27	0.94	0.79	0.51	0.15
b2	0.01	/	0.11	0.00	0.00	0.09	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00
b3	0.00	0.11	/	0.00	0.00	0.00	0.50	0.29	0.00	0.02	0.37	0.23	0.18	0.07	0.02	0.99	0.00
l1	0.00	0.00	0.00	/	0.00	0.00	0.88	0.00	0.00	0.48	0.02	0.02	0.00	0.17	0.02	0.37	0.00
l2	0.00	0.00	0.00	0.00	/	0.00	0.86	0.00	0.05	0.16	0.05	0.06	0.00	0.20	0.19	0.36	0.00
l3	0.00	0.09	0.00	0.00	0.00	/	0.64	0.14	0.00	0.05	0.44	0.11	0.16	0.06	0.01	0.97	0.00
MGHS	0.65	0.36	0.50	0.88	0.86	0.64	/	0.63	0.80	0.02	0.04	0.02	0.04	0.35	0.08	0.05	0.39
MeanGRB	0.00	0.00	0.29	0.00	0.00	0.14	0.63	/	0.00	0.82	0.08	0.00	0.00	0.01	0.00	0.36	0.00
Grmax	0.04	0.00	0.00	0.00	0.05	0.00	0.80	0.00	/	0.01	0.04	0.02	0.00	0.02	0.00	0.65	0.00
MGHT	0.70	0.00	0.02	0.48	0.16	0.05	0.02	0.82	0.01	/	0.02	0.00	0.00	0.00	0.01	0.07	0.00
MRW1	0.26	0.00	0.37	0.02	0.05	0.44	0.04	0.08	0.04	0.02	/	0.24	0.01	0.00	0.21	0.03	0.00
MRW2	0.39	0.00	0.23	0.02	0.06	0.11	0.02	0.00	0.02	0.00	0.24	/	0.00	0.00	0.00	0.00	0.00
MRW3	0.27	0.00	0.18	0.00	0.00	0.16	0.04	0.00	0.00	0.00	0.01	0.00	/	0.03	0.00	0.65	0.00
MPF1	0.94	0.00	0.07	0.17	0.20	0.06	0.35	0.01	0.02	0.00	0.00	0.00	0.03	/	0.00	0.00	0.00
MPF2	0.79	0.00	0.02	0.02	0.19	0.01	0.08	0.00	0.00	0.01	0.21	0.00	0.00	0.00	/	0.18	0.00
MU	0.51	0.23	0.99	0.37	0.36	0.97	0.05	0.36	0.65	0.07	0.03	0.00	0.65	0.00	0.18	/	0.30
MS	0.15	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	/

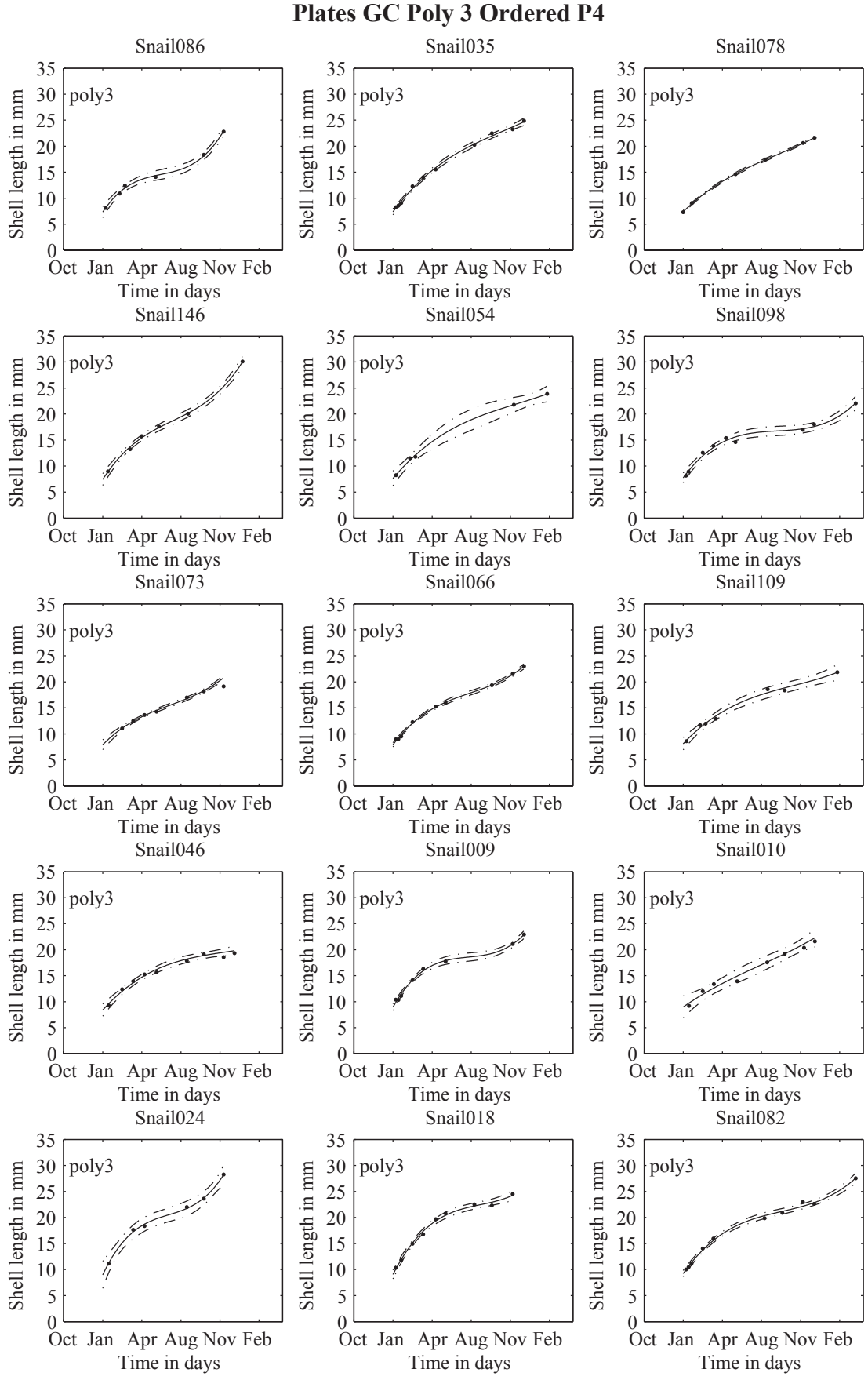
rho	b1	b2	b3	l1	l2	l3	MGHS	MeanGRB	Grmax	MGHT	MRW1	MRW2	MRW3	MPF1	MPF2	MU	MS
b1	/	0.28	-0.78	0.74	0.75	-0.72	-0.05	0.33	-0.21	0.04	0.12	-0.09	-0.12	0.01	0.03	0.07	0.15
b2	0.28	/	0.17	0.57	0.57	0.18	0.10	0.66	0.52	-0.43	0.35	-0.52	-0.55	0.43	0.48	0.13	0.91
b3	-0.78	0.17	/	-0.32	-0.61	0.97	0.07	0.11	0.72	-0.24	0.09	-0.13	-0.14	0.19	0.24	0.00	0.35
l1	0.74	0.57	-0.32	/	0.70	-0.32	-0.02	0.67	0.30	-0.07	0.24	-0.24	-0.34	0.14	0.24	0.09	0.58
l2	0.75	0.57	-0.61	0.70	/	-0.65	0.02	0.35	-0.20	-0.15	0.20	-0.20	-0.31	0.13	0.14	0.10	0.37
l3	-0.72	0.18	0.97	-0.32	-0.65	/	0.05	0.15	0.76	-0.21	0.08	-0.17	-0.15	0.20	0.27	0.00	0.37
MGHS	-0.05	0.10	0.07	-0.02	0.02	0.05	/	0.05	0.03	-0.25	0.21	-0.25	-0.22	0.10	0.18	0.21	0.09
MeanGRB	0.33	0.66	0.11	0.67	0.35	0.15	0.05	/	0.53	-0.02	0.18	-0.47	-0.51	0.27	0.44	0.10	0.84
Grmax	-0.21	0.52	0.72	0.30	-0.20	0.76	0.03	0.53	/	-0.25	0.22	-0.25	-0.33	0.24	0.40	0.05	0.70
MGHT	0.04	-0.43	-0.24	-0.07	-0.15	-0.21	-0.25	-0.02	-0.25	/	-0.24	0.32	0.33	-0.30	-0.28	-0.19	-0.31
MRW1	0.12	0.35	0.09	0.24	0.20	0.08	0.21	0.18	0.22	-0.24	/	-0.12	-0.28	0.33	0.13	0.23	0.31
MRW2	-0.09	-0.52	-0.13	-0.24	-0.20	-0.17	-0.25	-0.47	-0.25	0.32	-0.12	/	0.42	-0.53	-0.45	-0.43	-0.53
MRW3	-0.12	-0.55	-0.14	-0.34	-0.31	-0.15	-0.22	-0.51	-0.33	0.33	-0.28	0.42	/	-0.23	-0.66	-0.05	-0.58
MPF1	0.01	0.43	0.19	0.14	0.13	0.20	0.10	0.27	0.24	-0.30	0.33	-0.53	-0.23	/	0.47	0.37	0.42
MPF2	0.03	0.48	0.24	0.24	0.14	0.27	0.18	0.44	0.40	-0.28	0.13	-0.45	-0.66	0.47	/	0.14	0.52
MU	0.07	0.13	0.00	0.09	0.10	0.00	0.21	0.10	0.05	-0.19	0.23	-0.43	-0.05	0.37	0.14	/	0.11
MS	0.15	0.91	0.35	0.58	0.37	0.37	0.09	0.84	0.70	-0.31	0.31	-0.53	-0.58	0.42	0.52	0.11	/

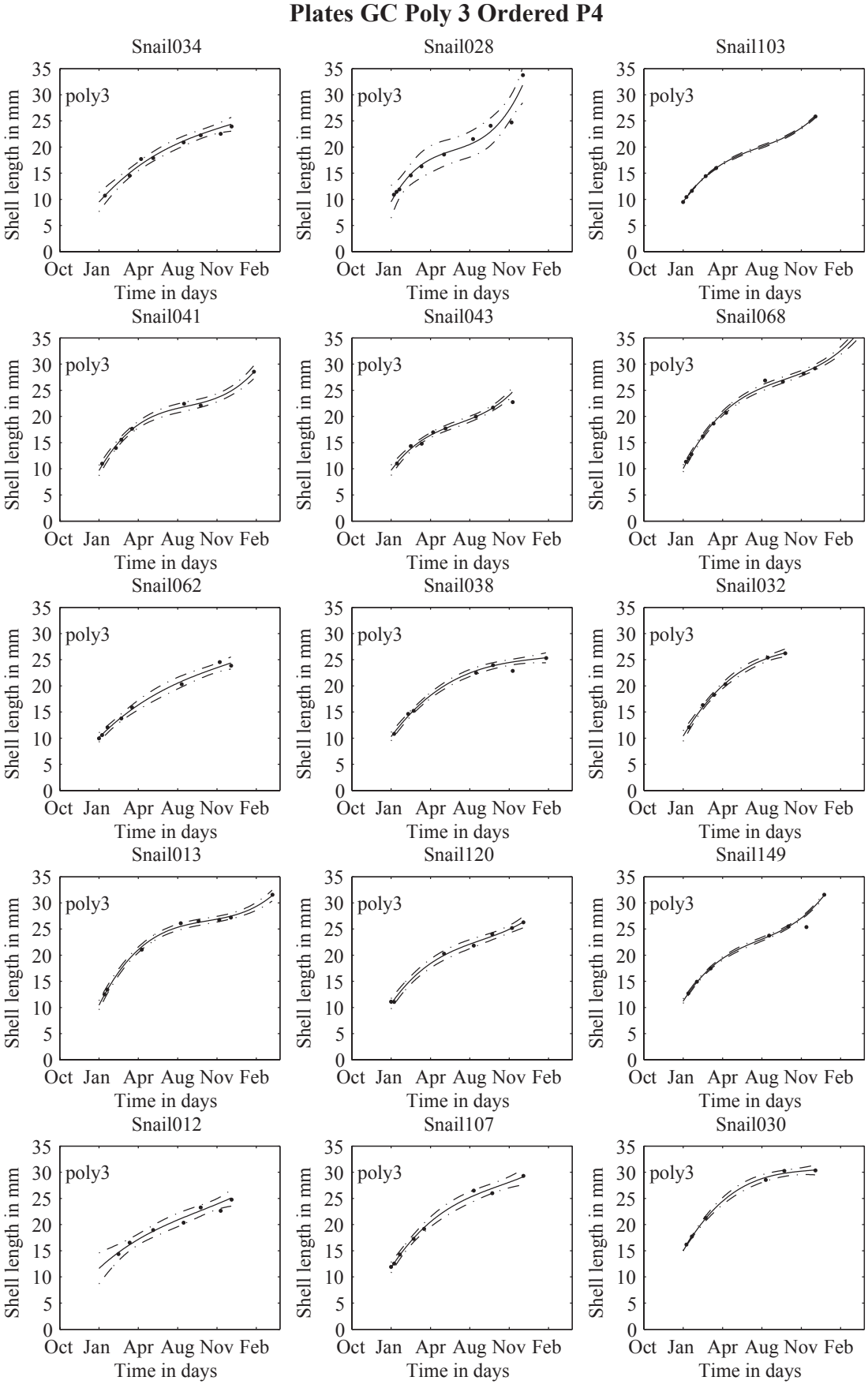

Plates GC Poly 3 Ordered P4
Snail201
Snail031
Snail067
Snail083
Snail088
Snail029
Snail006
Snail023
Snail136
Snail096
Snail111
Snail026
Snail056
Snail090
Snail119
poly1
Shell length in mm
Time in days
Oct Jan Apr Aug Nov Feb
0 5 10 15 20 25 30 35

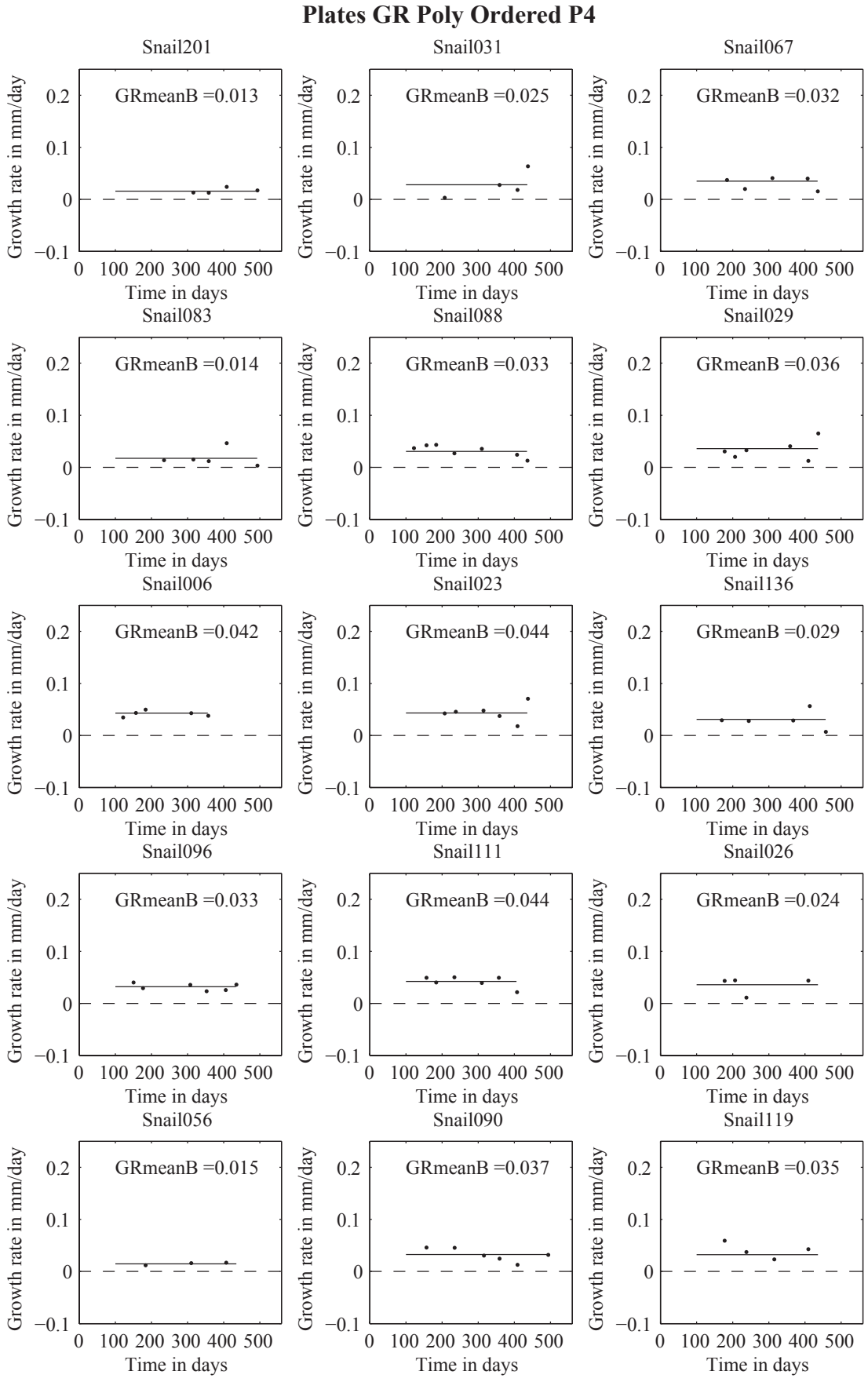

Plates GC Poly 3 Ordered P4
Snail065
poly1
Snail053
poly1
Snail063
poly1
Snail045
poly1
Snail153
poly2
Snail155
poly2
Snail156
poly2
Snail048
poly2
Snail104
poly2
Snail036
poly2
Snail008
poly2
Snail142
poly3
Snail152
poly3
Snail061
poly3
Snail140
poly3
Shell length in mm
0 5 10 15 20 25 30 35
Oct Jan Apr Aug Nov Feb
Time in days

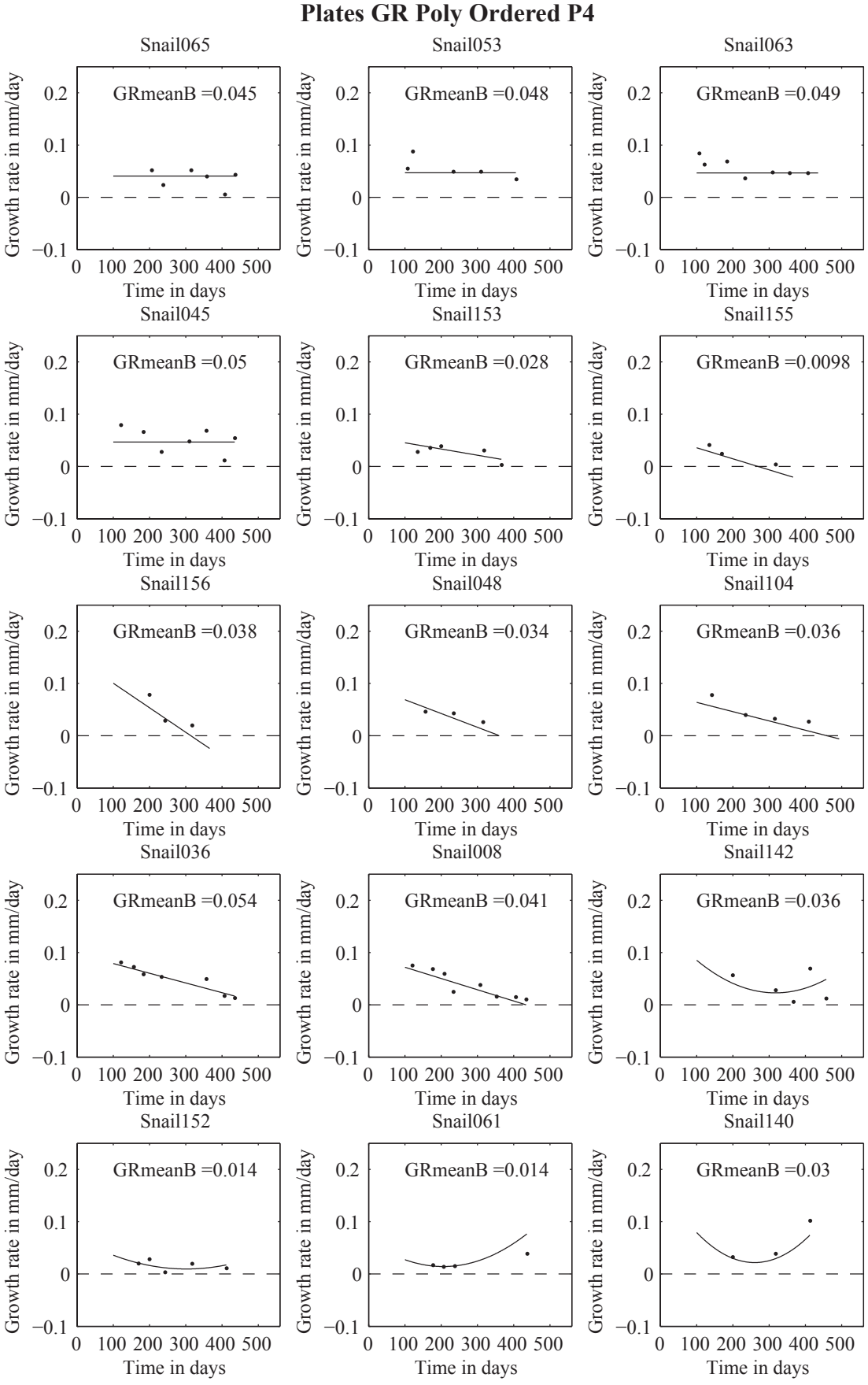

Plates GC Poly 3 Ordered P4
Snail144
Snail150
Snail141
Snail131
Snail130
Snail147
Snail064
Snail145
Snail021
Snail007
Snail058
Snail133
Snail134
Snail002
Snail100
poly3
Shell length in mm
Time in days
Oct Jan Apr Aug Nov Feb
0 5 10 15 20 25 30 35

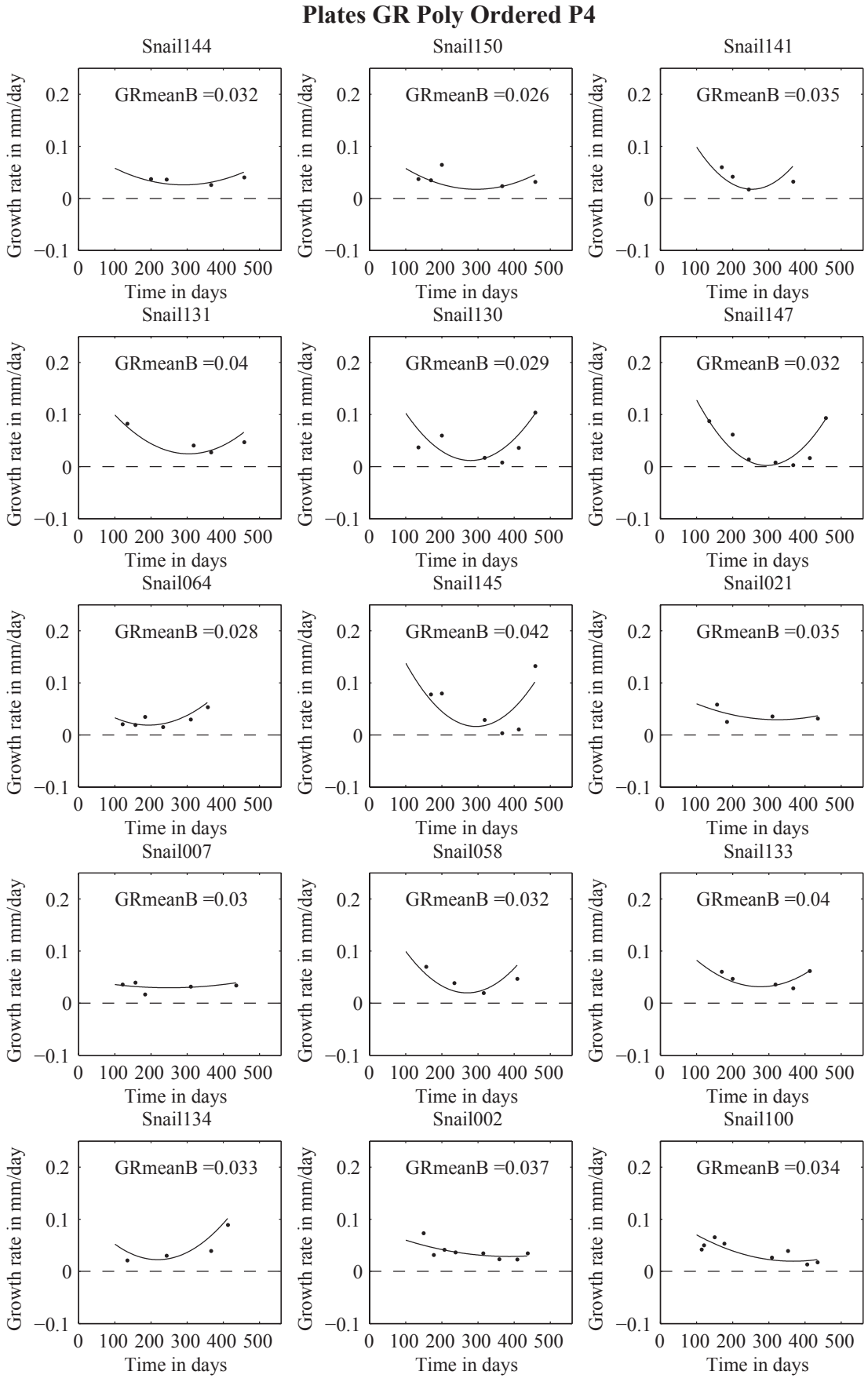

Plates GC Poly 3 Ordered P4
Snail112
Snail052
Snail071
Snail057
Snail143
Snail139
Snail047
Snail148
Snail055
Snail095
Snail116
Snail022
Snail072
Snail044
Snail092
poly3
Shell length in mm
0 5 10 15 20 25 30 35
Oct Jan Apr Aug Nov Feb
Time in days

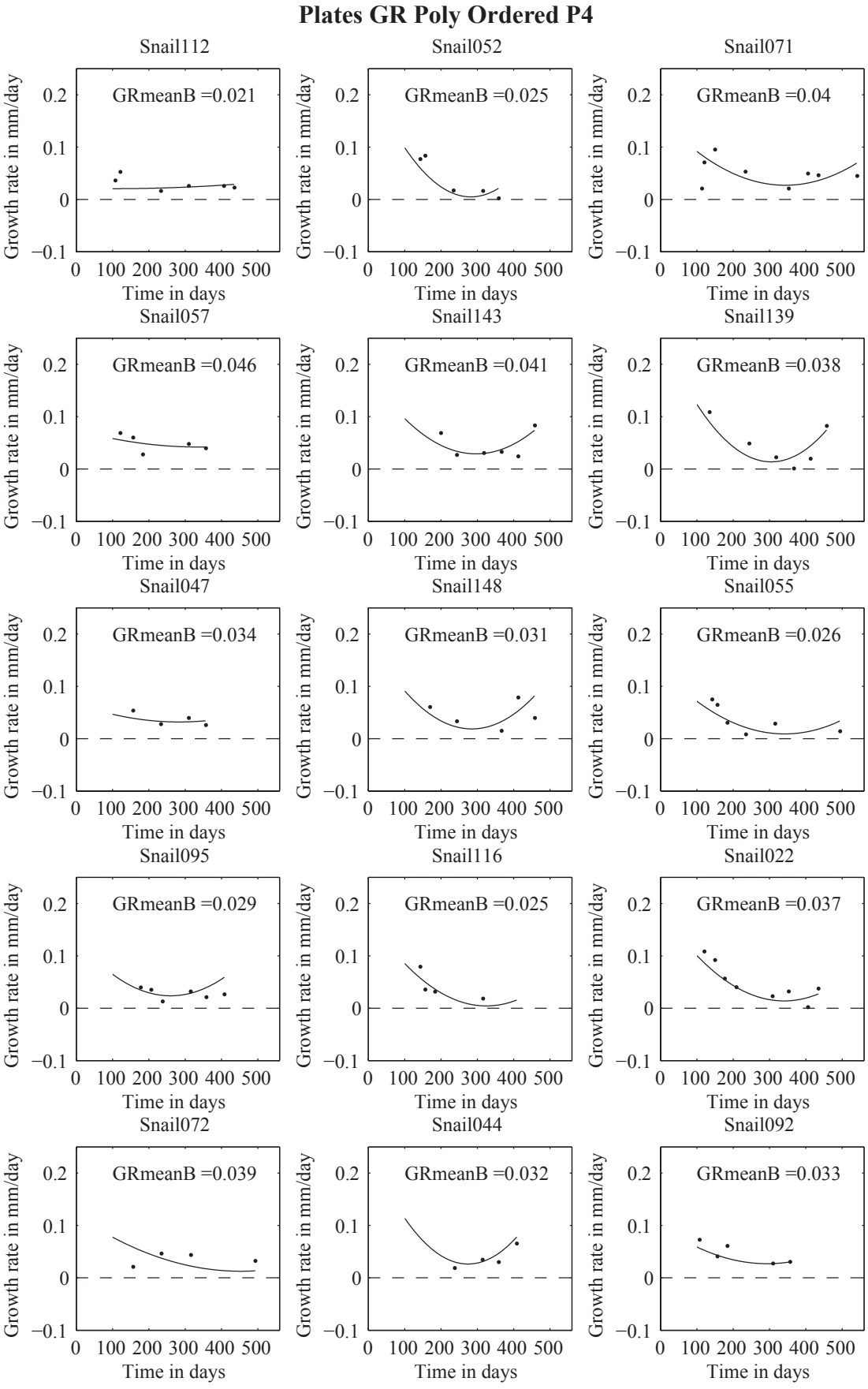

Plates GC Poly 3 Ordered P4
Snail086
Snail035
Snail078
Snail146
Snail054
Snail098
Snail073
Snail066
Snail109
Snail046
Snail009
Snail010
Snail024
Snail018
Snail082
poly3
Shell length in mm
0 5 10 15 20 25 30 35
Oct Jan Apr Aug Nov Feb
Time in days

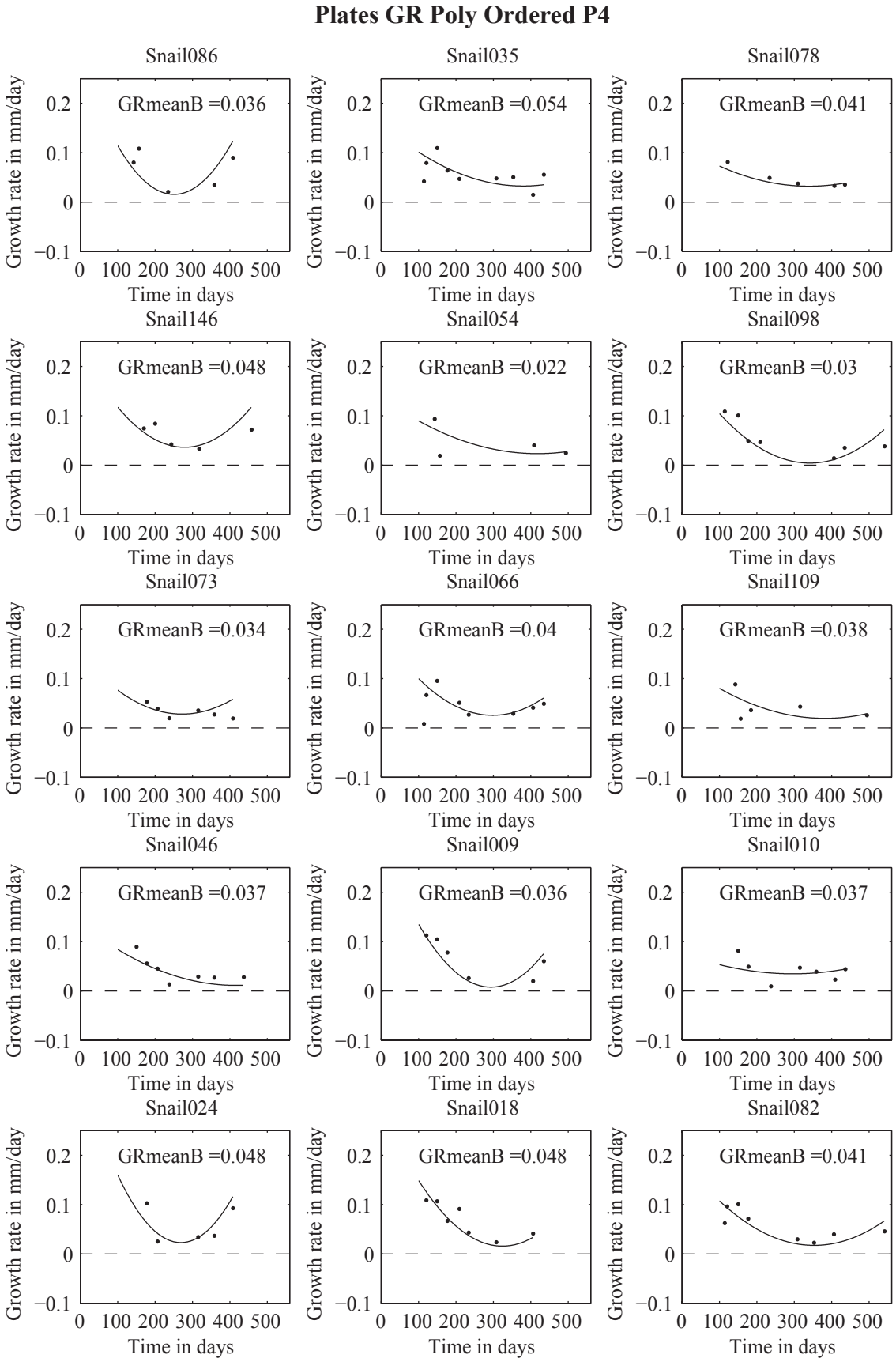

Plates GC Poly 3 Ordered P4
Snail034
Snail028
Snail103
Snail041
Snail043
Snail068
Snail062
Snail038
Snail032
Snail013
Snail120
Snail149
Snail012
Snail107
Snail030
poly3
Shell length in mm
0 5 10 15 20 25 30 35
Oct Jan Apr Aug Nov Feb
Time in days

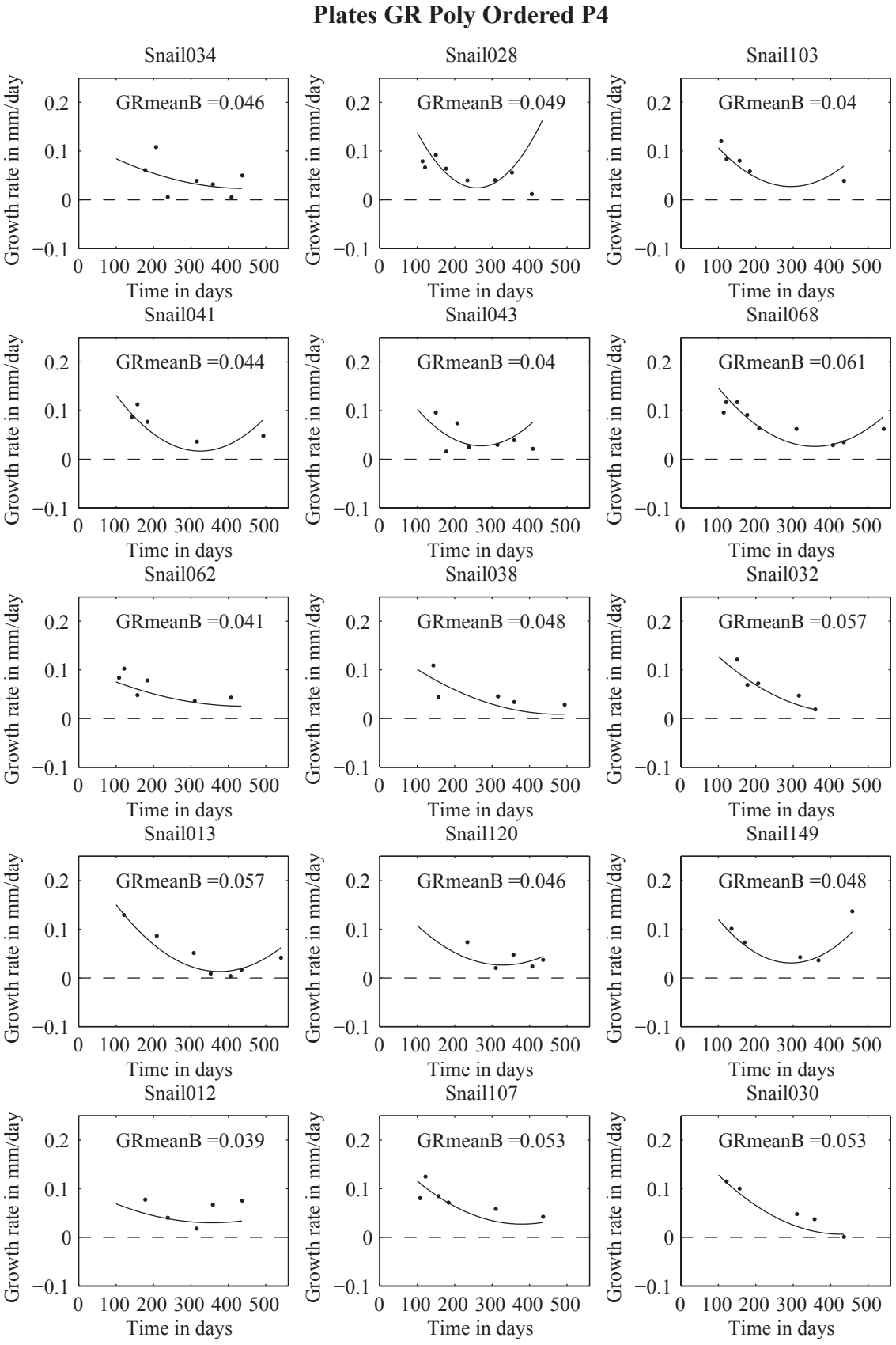

Plates GR Poly Ordered P4
Snail201
GRmeanB =0.013
Snail031
GRmeanB =0.025
Snail067
GRmeanB =0.032
Snail083
GRmeanB =0.014
Snail088
GRmeanB =0.033
Snail029
GRmeanB =0.036
Snail006
GRmeanB =0.042
Snail023
GRmeanB =0.044
Snail136
GRmeanB =0.029
Snail096
GRmeanB =0.033
Snail111
GRmeanB =0.044
Snail026
GRmeanB =0.024
Snail056
GRmeanB =0.015
Snail090
GRmeanB =0.037
Snail119
GRmeanB =0.035
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

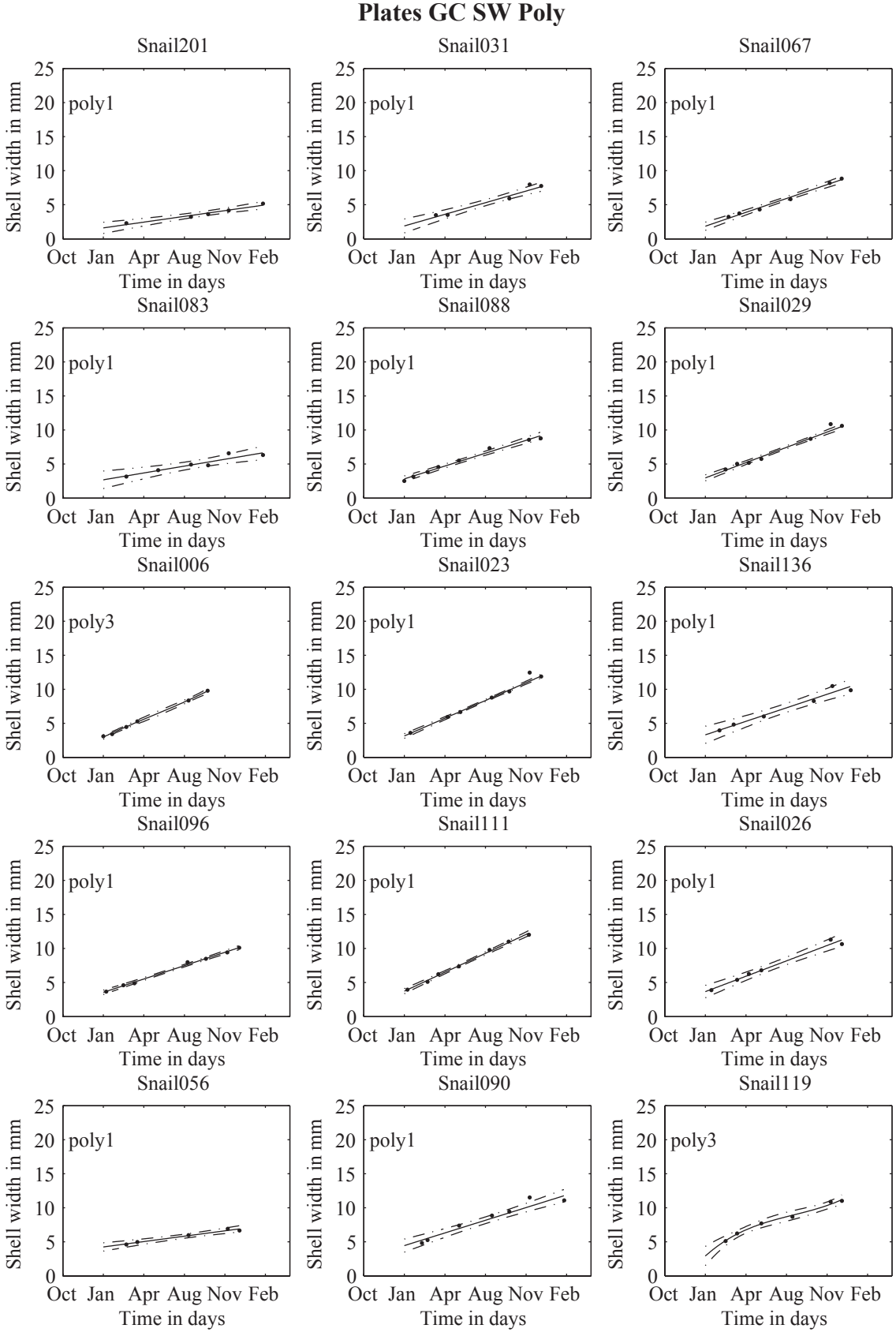

Plates GR Poly Ordered P4
Snail065
GRmeanB =0.045
Snail053
GRmeanB =0.048
Snail063
GRmeanB =0.049
Snail045
GRmeanB =0.05
Snail153
GRmeanB =0.028
Snail155
GRmeanB =0.0098
Snail156
GRmeanB =0.038
Snail048
GRmeanB =0.034
Snail104
GRmeanB =0.036
Snail036
GRmeanB =0.054
Snail008
GRmeanB =0.041
Snail142
GRmeanB =0.036
Snail152
GRmeanB =0.014
Snail061
GRmeanB =0.014
Snail140
GRmeanB =0.03
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

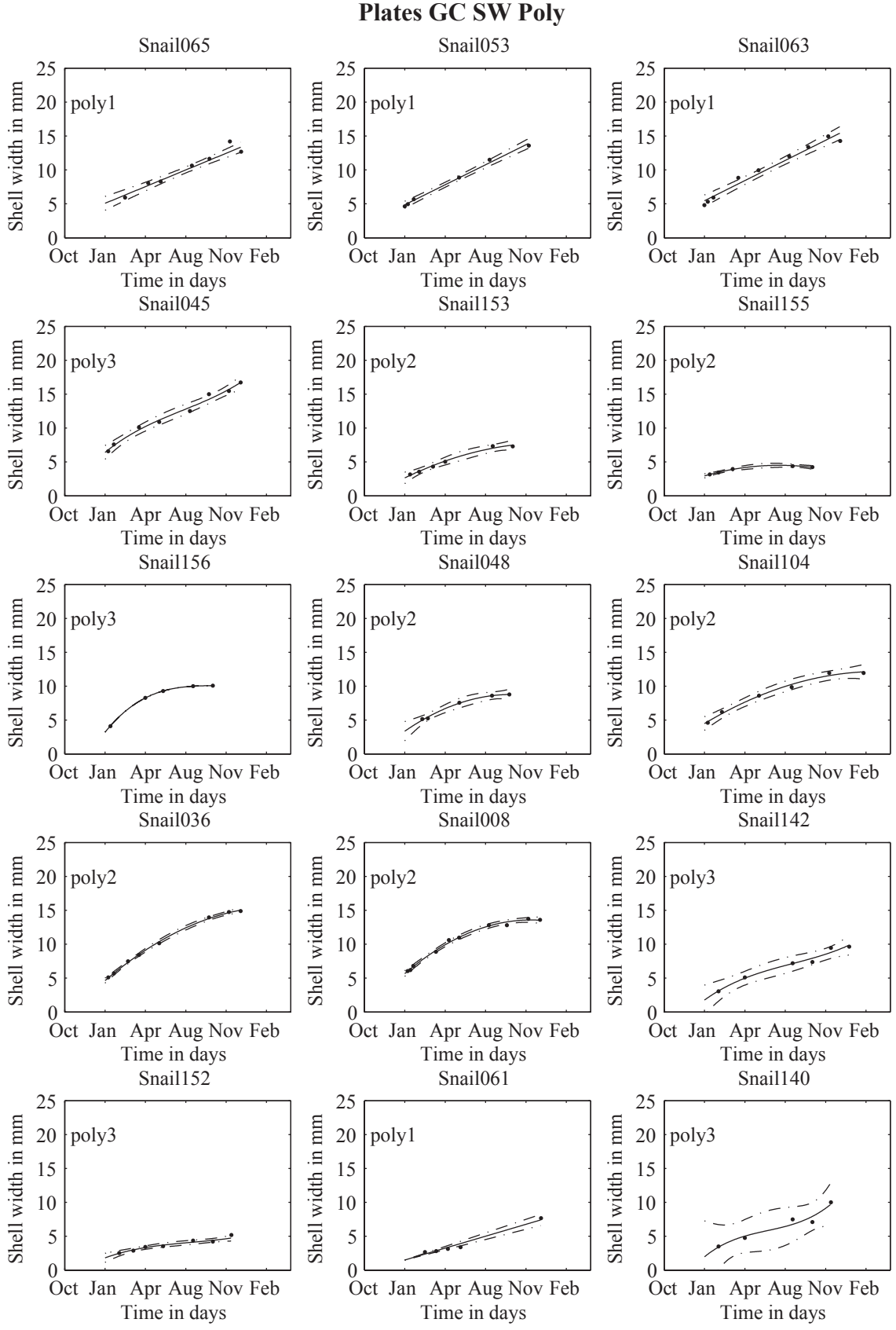

Plates GR Poly Ordered P4
Snail144
GRmeanB =0.032
Snail150
GRmeanB =0.026
Snail141
GRmeanB =0.035
Snail131
GRmeanB =0.04
Snail130
GRmeanB =0.029
Snail147
GRmeanB =0.032
Snail064
GRmeanB =0.028
Snail145
GRmeanB =0.042
Snail021
GRmeanB =0.035
Snail007
GRmeanB =0.03
Snail058
GRmeanB =0.032
Snail133
GRmeanB =0.04
Snail134
GRmeanB =0.033
Snail002
GRmeanB =0.037
Snail100
GRmeanB =0.034
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

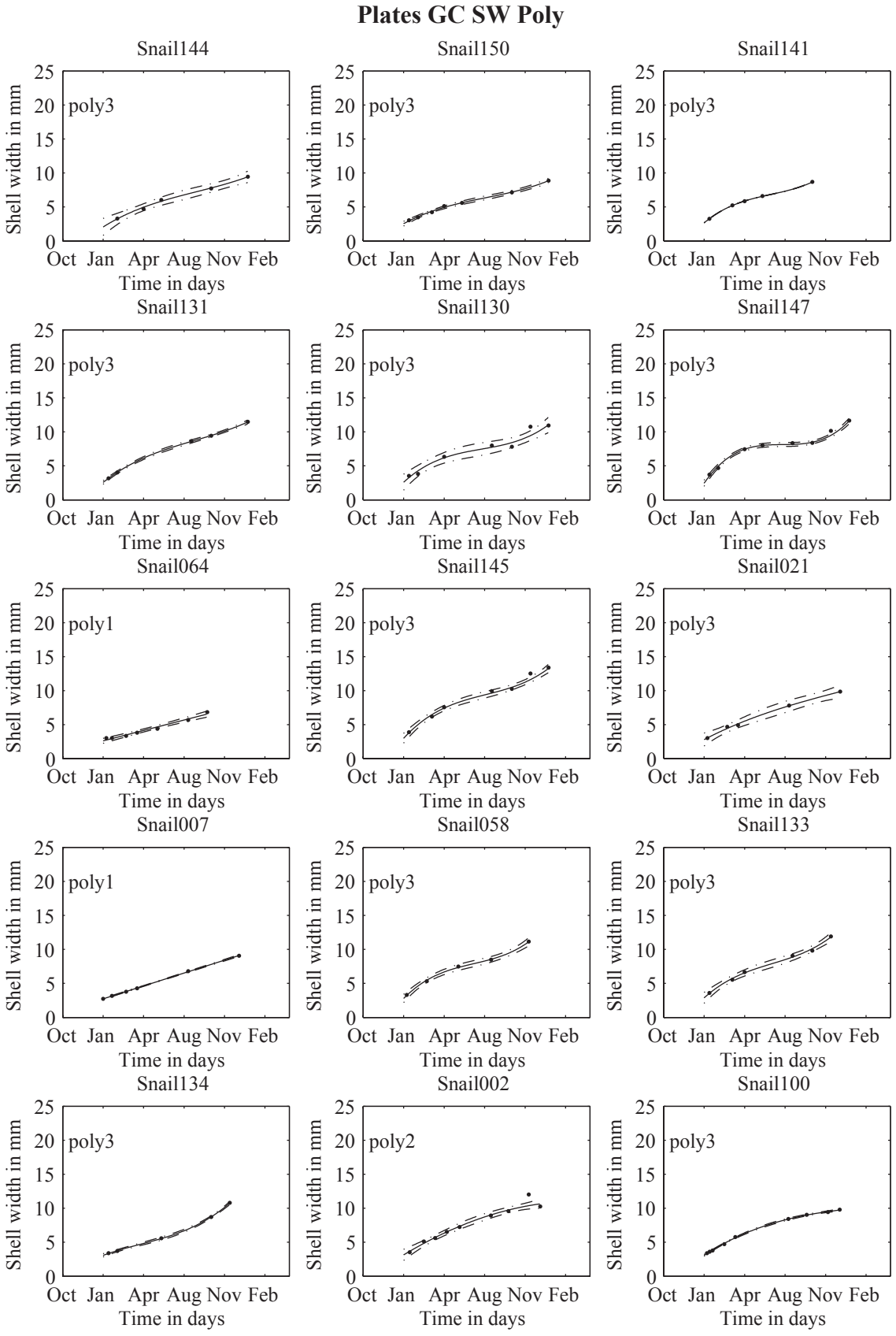

Plates GR Poly Ordered P4
Snail112
GRmeanB =0.021
Snail052
GRmeanB =0.025
Snail071
GRmeanB =0.04
Snail057
GRmeanB =0.046
Snail143
GRmeanB =0.041
Snail139
GRmeanB =0.038
Snail047
GRmeanB =0.034
Snail148
GRmeanB =0.031
Snail055
GRmeanB =0.026
Snail095
GRmeanB =0.029
Snail116
GRmeanB =0.025
Snail022
GRmeanB =0.037
Snail072
GRmeanB =0.039
Snail044
GRmeanB =0.032
Snail092
GRmeanB =0.033
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

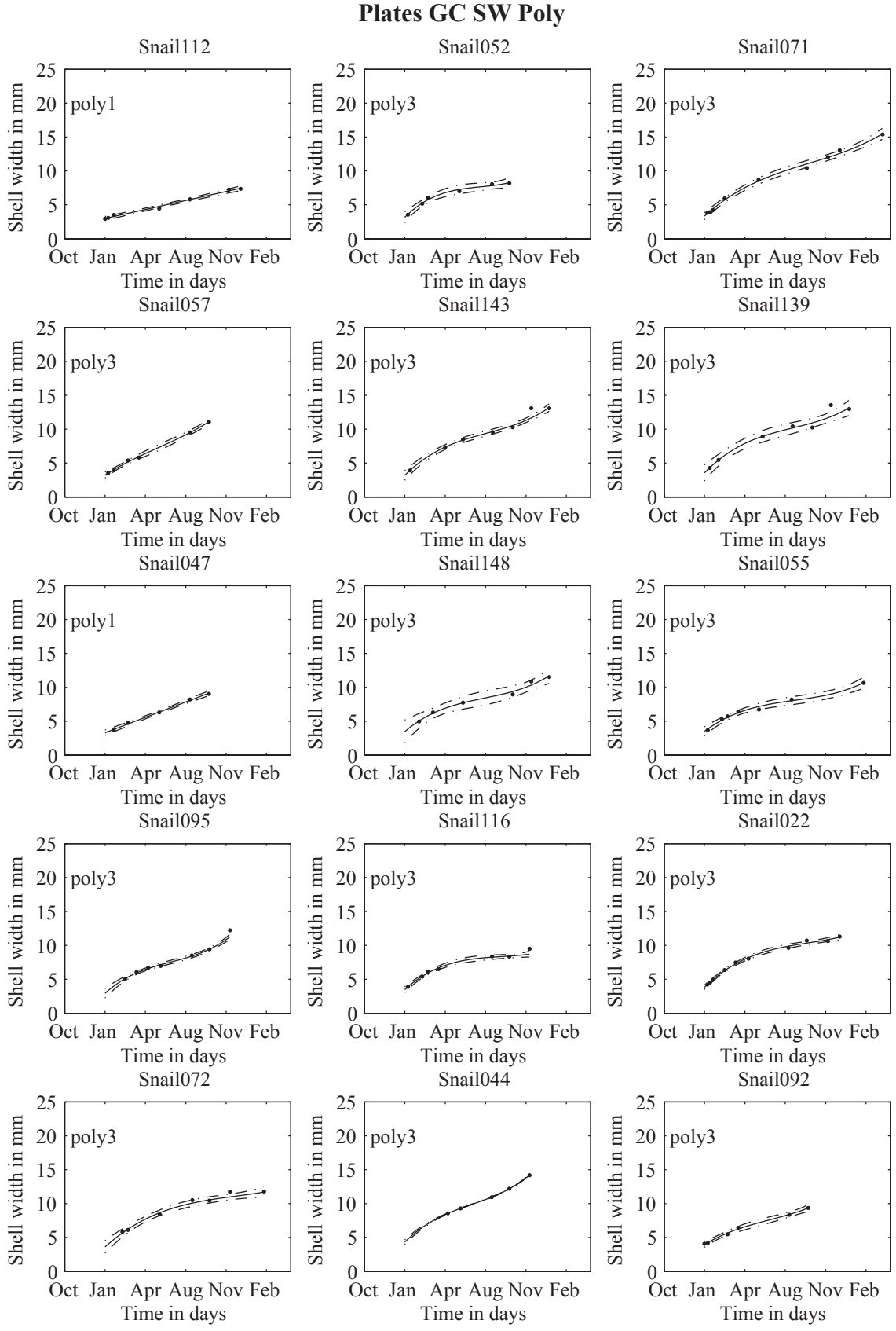

Plates GR Poly Ordered P4
Snail086
GRmeanB =0.036
Snail035
GRmeanB =0.054
Snail078
GRmeanB =0.041
Snail146
GRmeanB =0.048
Snail054
GRmeanB =0.022
Snail098
GRmeanB =0.03
Snail073
GRmeanB =0.034
Snail066
GRmeanB =0.04
Snail109
GRmeanB =0.038
Snail046
GRmeanB =0.037
Snail009
GRmeanB =0.036
Snail010
GRmeanB =0.037
Snail024
GRmeanB =0.048
Snail018
GRmeanB =0.048
Snail082
GRmeanB =0.041
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

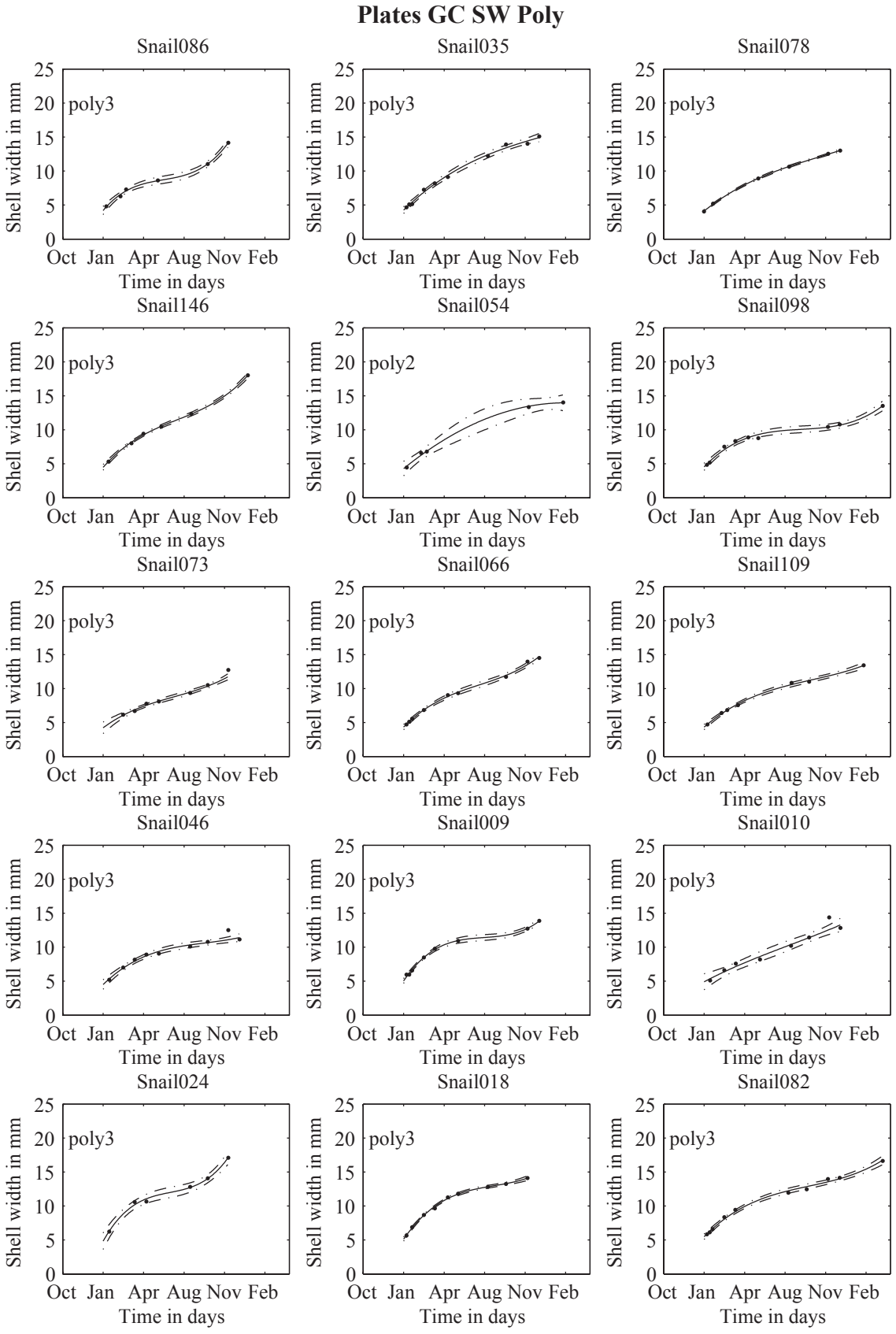

Plates GR Poly Ordered P4
Snail034
GRmeanB =0.046
Snail028
GRmeanB =0.049
Snail103
GRmeanB =0.04
Snail041
GRmeanB =0.044
Snail043
GRmeanB =0.04
Snail068
GRmeanB =0.061
Snail062
GRmeanB =0.041
Snail038
GRmeanB =0.048
Snail032
GRmeanB =0.057
Snail013
GRmeanB =0.057
Snail120
GRmeanB =0.046
Snail149
GRmeanB =0.048
Snail012
GRmeanB =0.039
Snail107
GRmeanB =0.053
Snail030
GRmeanB =0.053
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

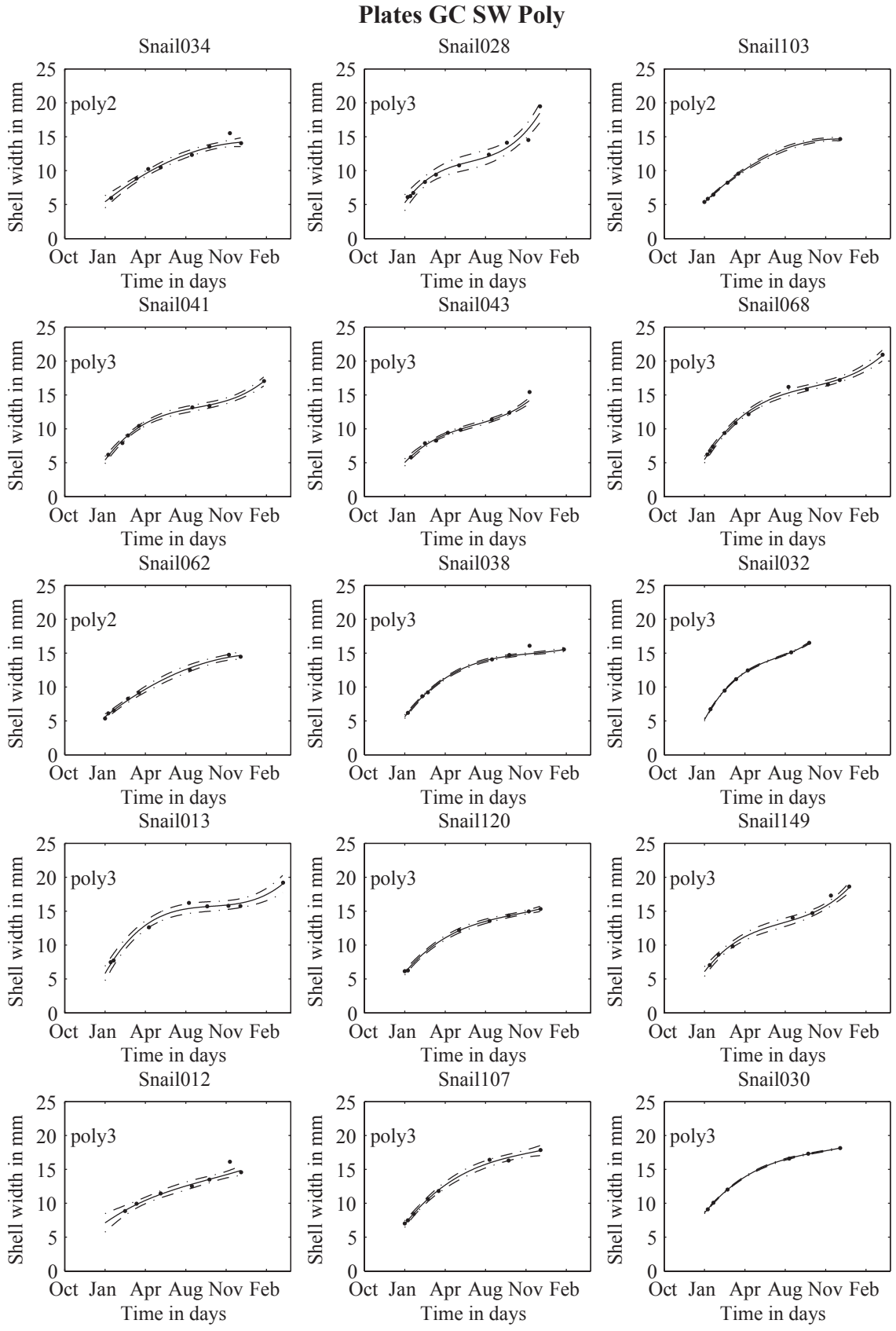

Plates GC SW Poly
Snail201
poly1
Snail031
poly1
Snail067
poly1
Snail083
poly1
Snail088
poly1
Snail029
poly1
Snail006
poly3
Snail023
poly1
Snail136
poly1
Snail096
poly1
Snail111
poly1
Snail026
poly1
Snail056
poly1
Snail090
poly1
Snail119
poly3
Shell width in mm
0 5 10 15 20 25
Oct Jan Apr Aug Nov Feb
Time in days


Plates GC SW Poly
Snail065
poly1
Snail053
poly1
Snail063
poly1
Snail045
poly3
Snail153
poly2
Snail155
poly2
Snail156
poly3
Snail048
poly2
Snail104
poly2
Snail036
poly2
Snail008
poly2
Snail142
poly3
Snail152
poly3
Snail061
poly1
Snail140
poly3
Shell width in mm
0 5 10 15 20 25
Oct Jan Apr Aug Nov Feb
Time in days

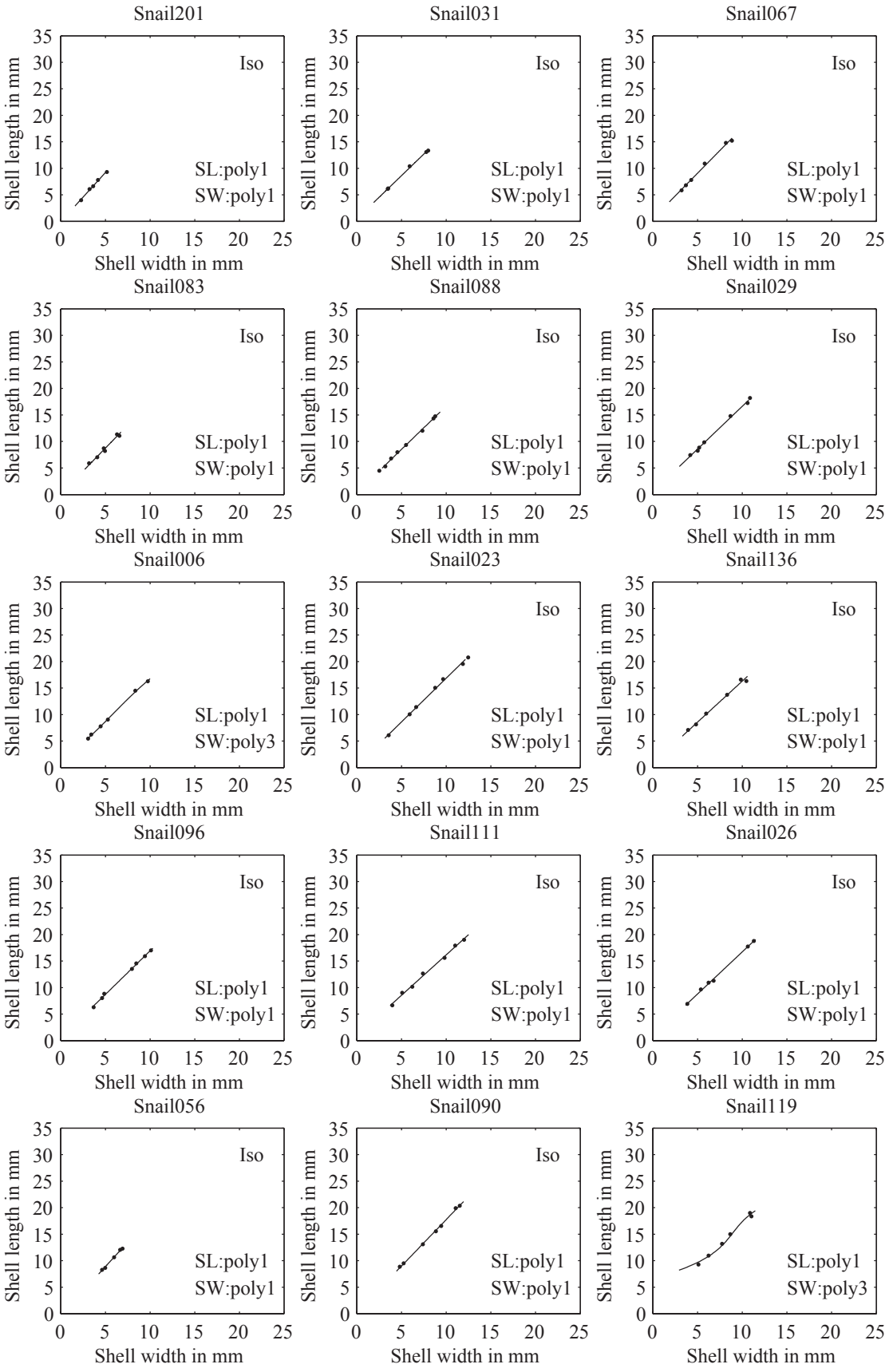

Plates GC SW Poly
Snail144
poly3
Snail150
poly3
Snail141
poly3
Snail131
poly3
Snail130
poly3
Snail147
poly3
Snail064
poly1
Snail145
poly3
Snail021
poly3
Snail007
poly1
Snail058
poly3
Snail133
poly3
Snail134
poly3
Snail002
poly2
Snail100
poly3
Shell width in mm
0 5 10 15 20 25
Oct Jan Apr Aug Nov Feb
Time in days

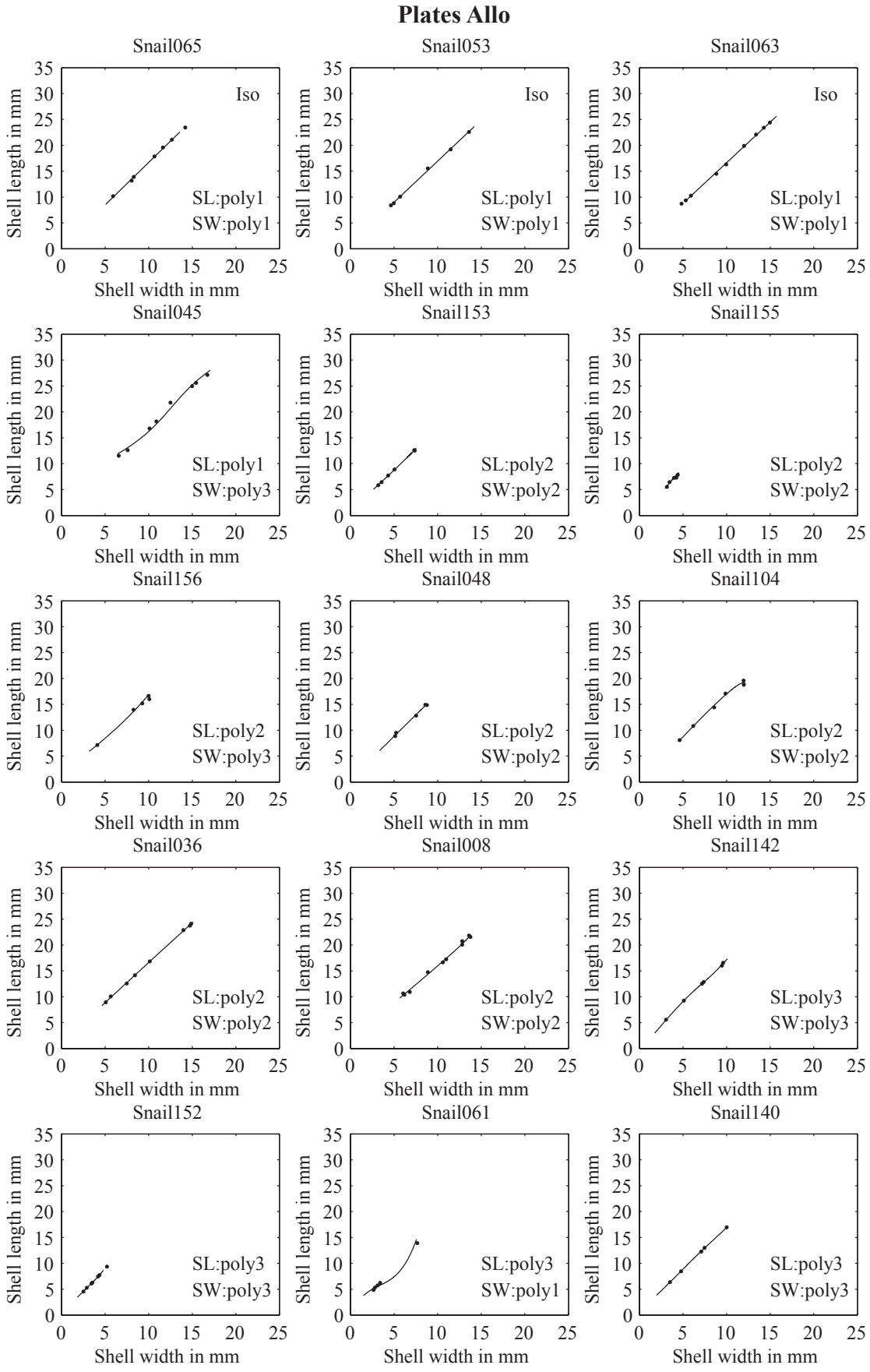

Plates GC SW Poly
Snail112
poly1
Snail052
poly3
Snail071
poly3
Snail057
poly3
Snail143
poly3
Snail139
poly3
Snail047
poly1
Snail148
poly3
Snail055
poly3
Snail095
poly3
Snail116
poly3
Snail022
poly3
Snail072
poly3
Snail044
poly3
Snail092
poly3
Shell width in mm
Time in days
Oct Jan Apr Aug Nov Feb
0 5 10 15 20 25


Plates GC SW Poly
Snail086
Snail035
Snail078
Snail146
Snail054
Snail098
Snail073
Snail066
Snail109
Snail046
Snail009
Snail010
Snail024
Snail018
Snail082
poly3
poly2
Shell width in mm
Time in days
Oct Jan Apr Aug Nov Feb
0 5 10 15 20 25

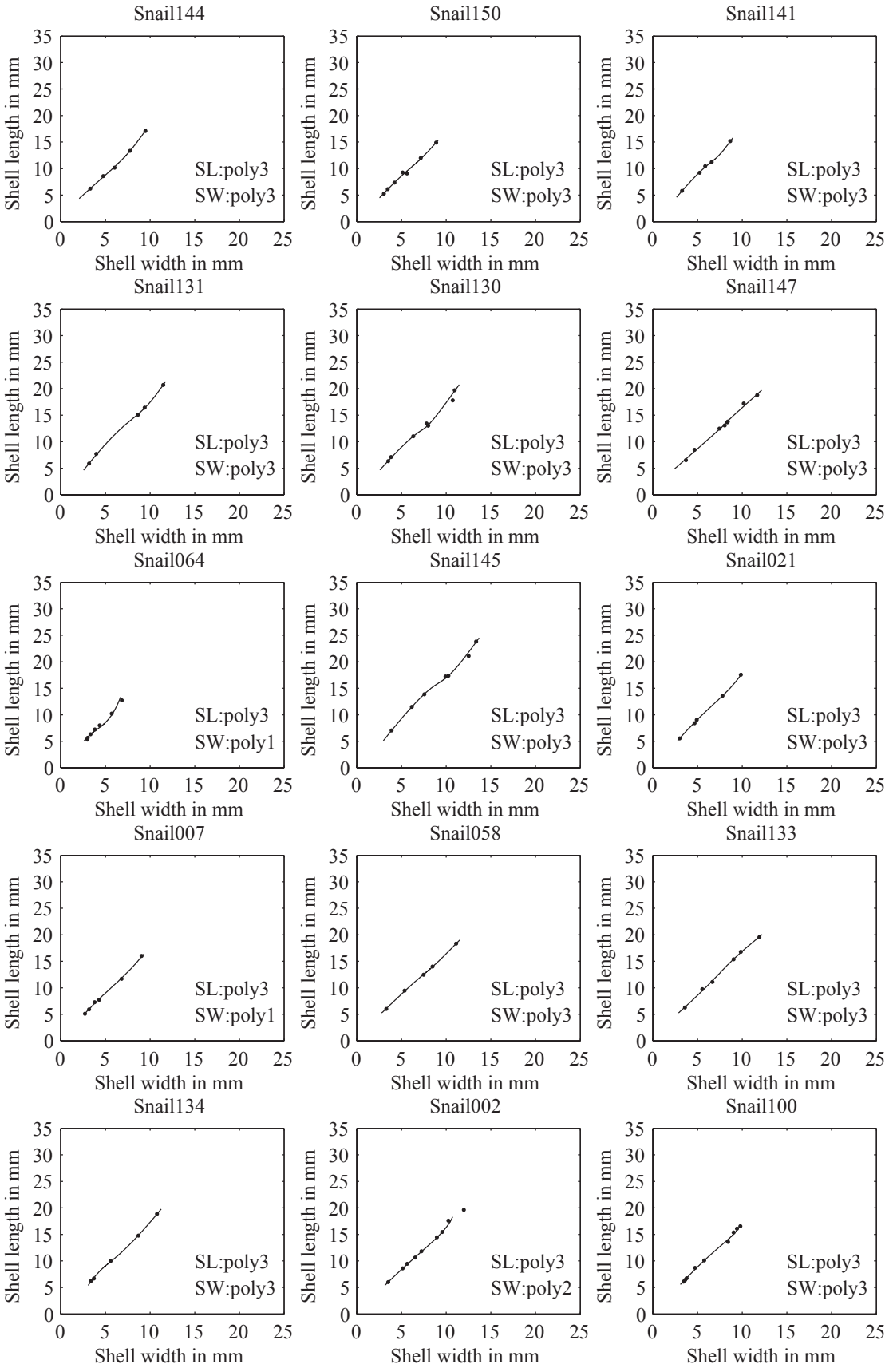

Plates GC SW Poly
Snail034
poly2
Snail028
poly3
Snail103
poly2
Snail041
poly3
Snail043
poly3
Snail068
poly3
Snail062
poly2
Snail038
poly3
Snail032
poly3
Snail013
poly3
Snail120
poly3
Snail149
poly3
Snail012
poly3
Snail107
poly3
Snail030
poly3
Shell width in mm
Time in days
Oct Jan Apr Aug Nov Feb
0 5 10 15 20 25

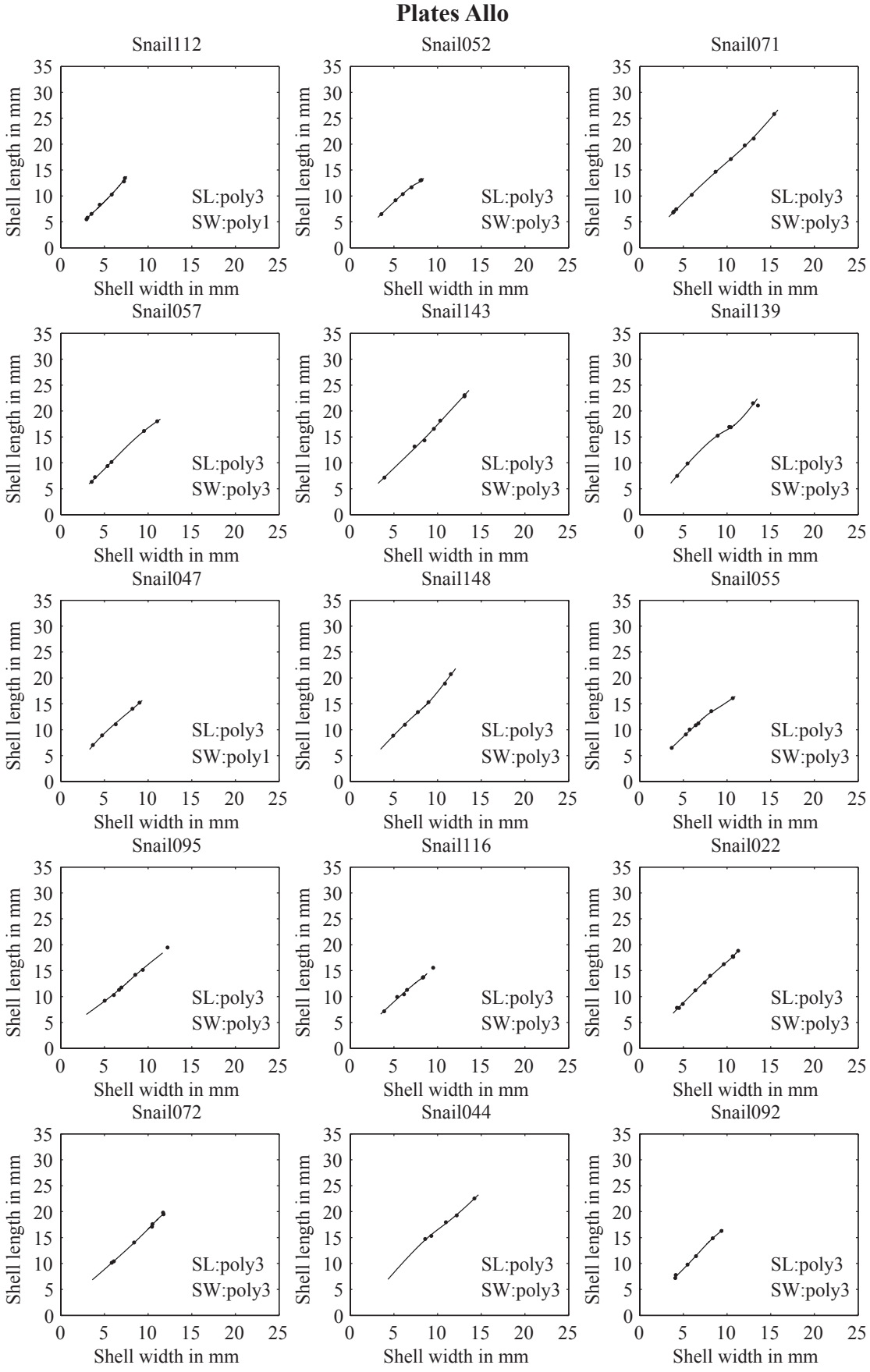
Plates Allo

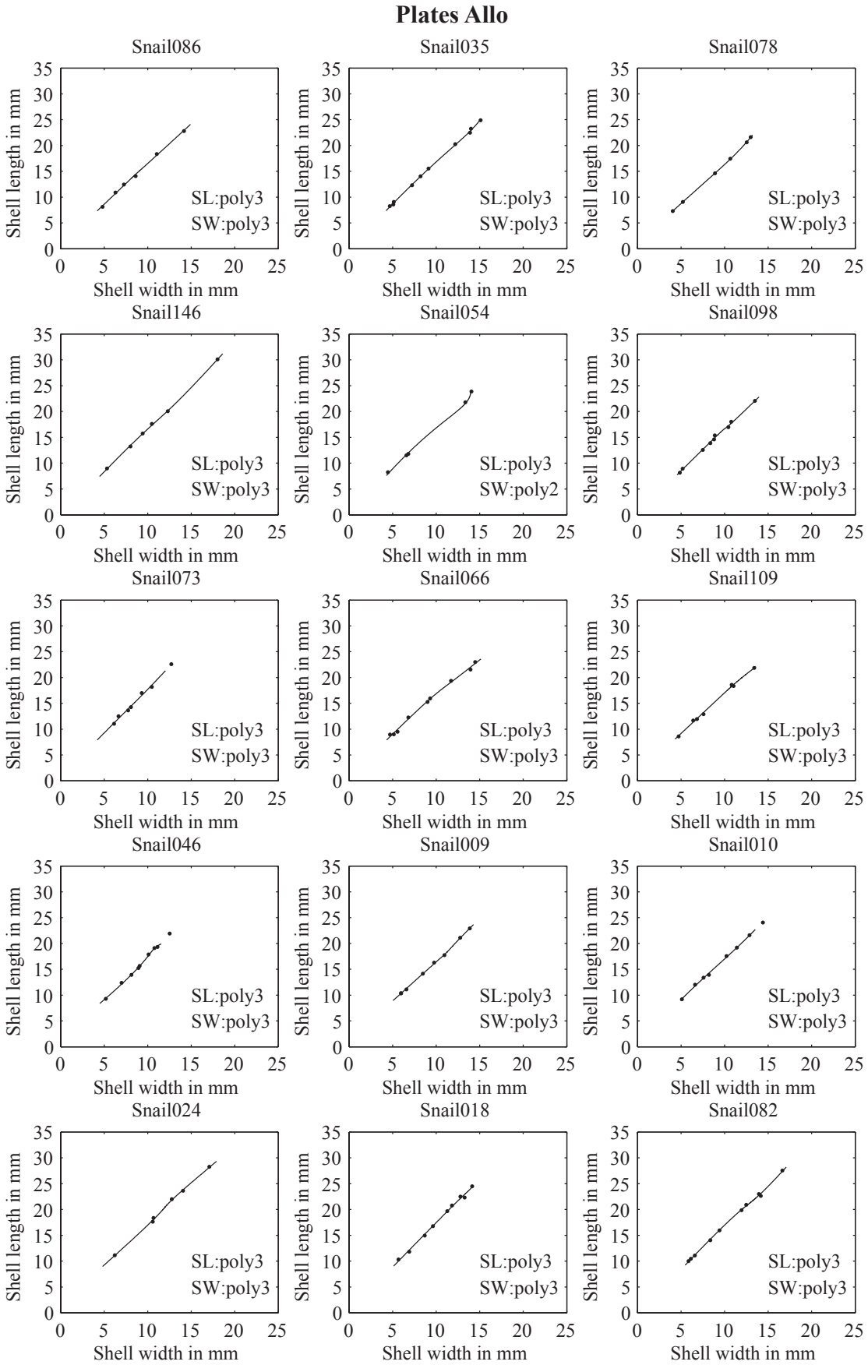

Snail201
Iso
SL:poly1
SW:poly1
Snail031
Iso
SL:poly1
SW:poly1
Snail067
Iso
SL:poly1
SW:poly1
Snail083
Iso
SL:poly1
SW:poly1
Snail088
Iso
SL:poly1
SW:poly1
Snail029
Iso
SL:poly1
SW:poly1
Snail006
Iso
SL:poly1
SW:poly3
Snail023
Iso
SL:poly1
SW:poly1
Snail136
Iso
SL:poly1
SW:poly1
Snail096
Iso
SL:poly1
SW:poly1
Snail111
Iso
SL:poly1
SW:poly1
Snail026
Iso
SL:poly1
SW:poly1
Snail056
Iso
SL:poly1
SW:poly1
Snail090
Iso
SL:poly1
SW:poly1
Snail119
Iso
SL:poly1
SW:poly3
Shell length in mm
Shell width in mm
0 5 10 15 20 25
0 5 10 15 20 25 30 35

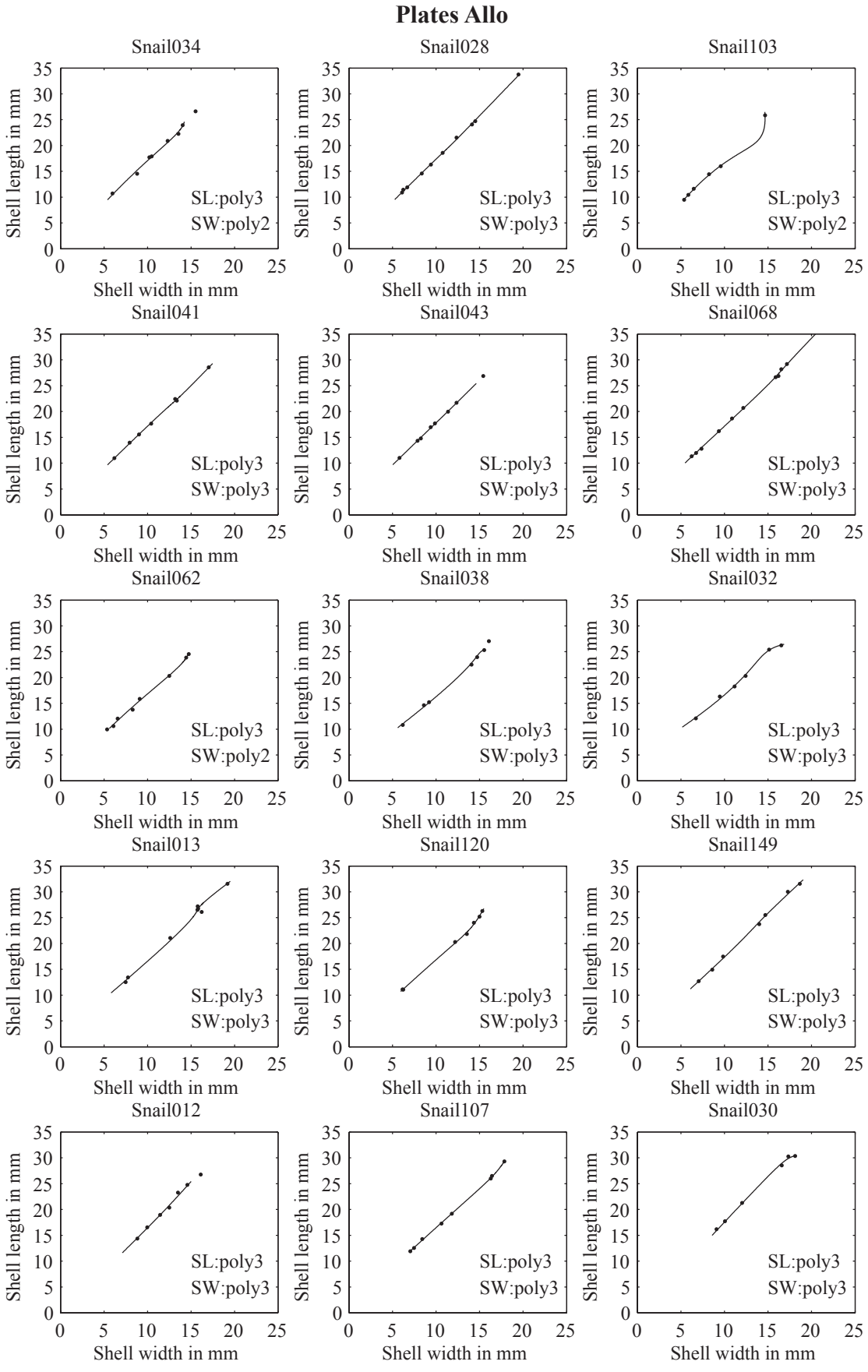

Plates Allo
Snail065
Iso
SL:poly1
SW:poly1
Snail053
Iso
SL:poly1
SW:poly1
Snail063
Iso
SL:poly1
SW:poly1
Snail045
SL:poly1
SW:poly3
Snail153
SL:poly2
SW:poly2
Snail155
SL:poly2
SW:poly2
Snail156
SL:poly2
SW:poly3
Snail048
SL:poly2
SW:poly2
Snail104
SL:poly2
SW:poly2
Snail036
SL:poly2
SW:poly2
Snail008
SL:poly2
SW:poly2
Snail142
SL:poly3
SW:poly3
Snail152
SL:poly3
SW:poly3
Snail061
SL:poly3
SW:poly1
Snail140
SL:poly3
SW:poly3
Shell length in mm
Shell width in mm

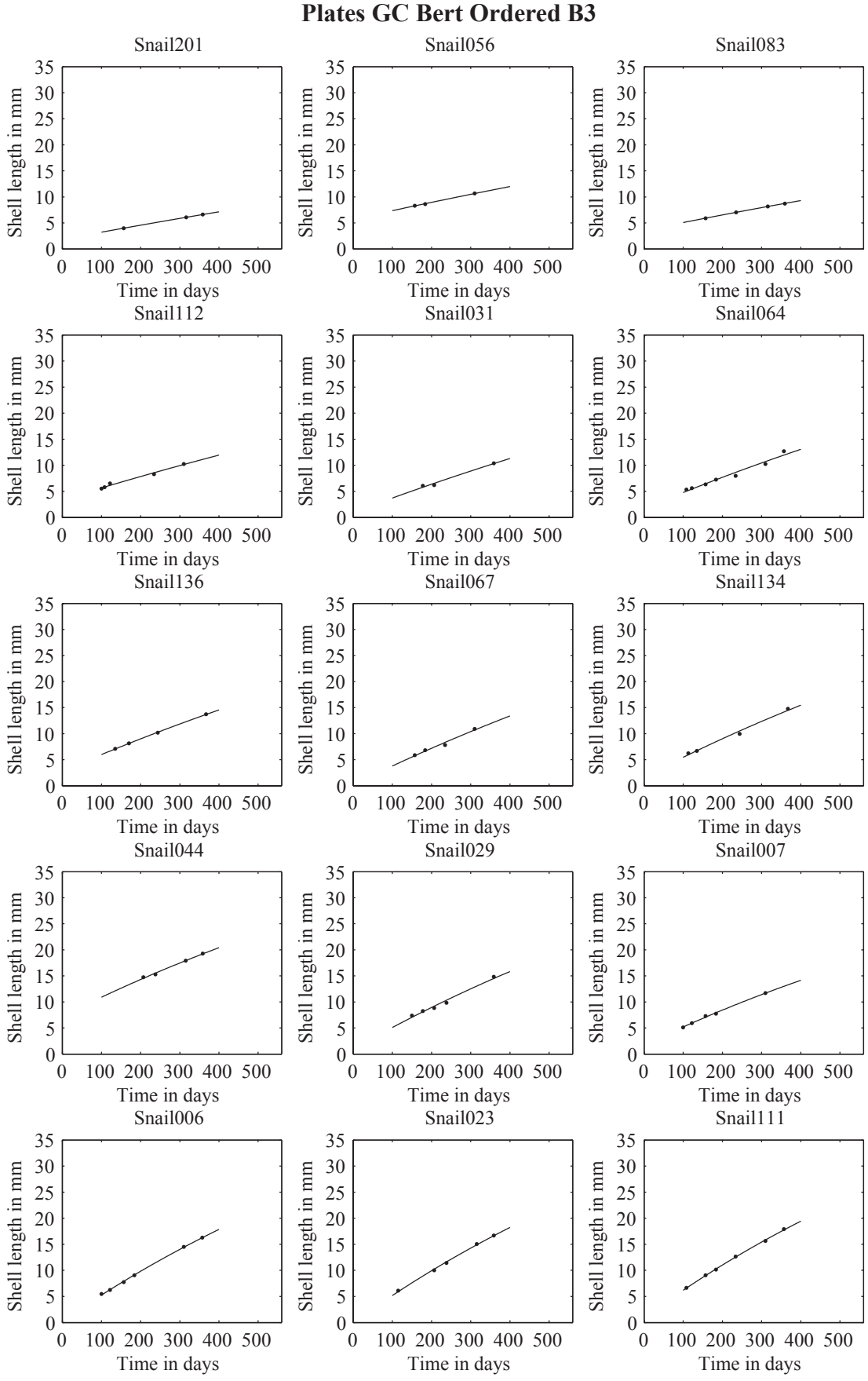
Plates Allo

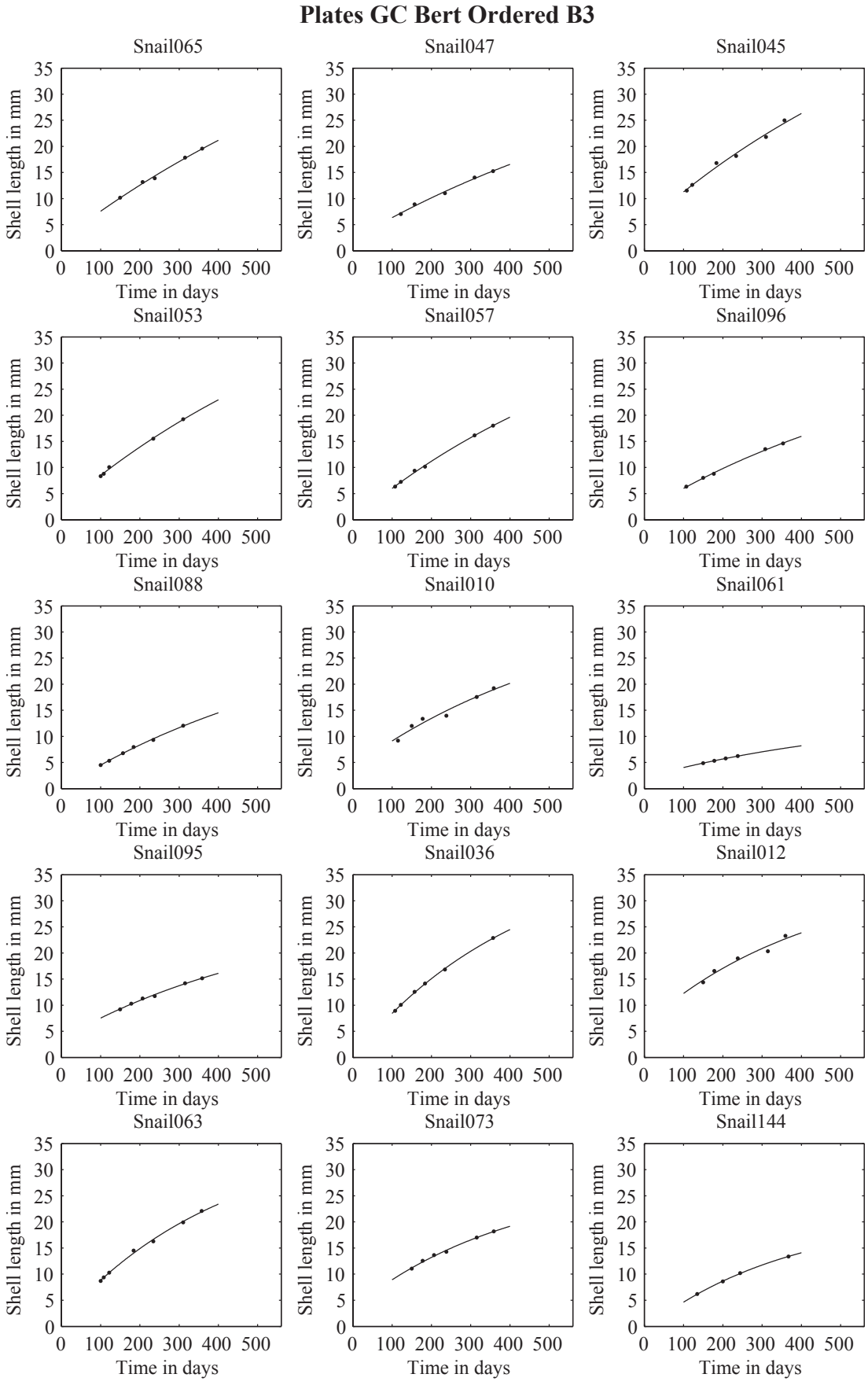

Snail144
SL:poly3
SW:poly3
Snail150
SL:poly3
SW:poly3
Snail141
SL:poly3
SW:poly3
Snail131
SL:poly3
SW:poly3
Snail130
SL:poly3
SW:poly3
Snail147
SL:poly3
SW:poly3
Snail064
SL:poly3
SW:poly1
Snail145
SL:poly3
SW:poly3
Snail021
SL:poly3
SW:poly3
Snail007
SL:poly3
SW:poly1
Snail058
SL:poly3
SW:poly3
Snail133
SL:poly3
SW:poly3
Snail134
SL:poly3
SW:poly3
Snail002
SL:poly3
SW:poly2
Snail100
SL:poly3
SW:poly3
Shell length in mm
Shell width in mm

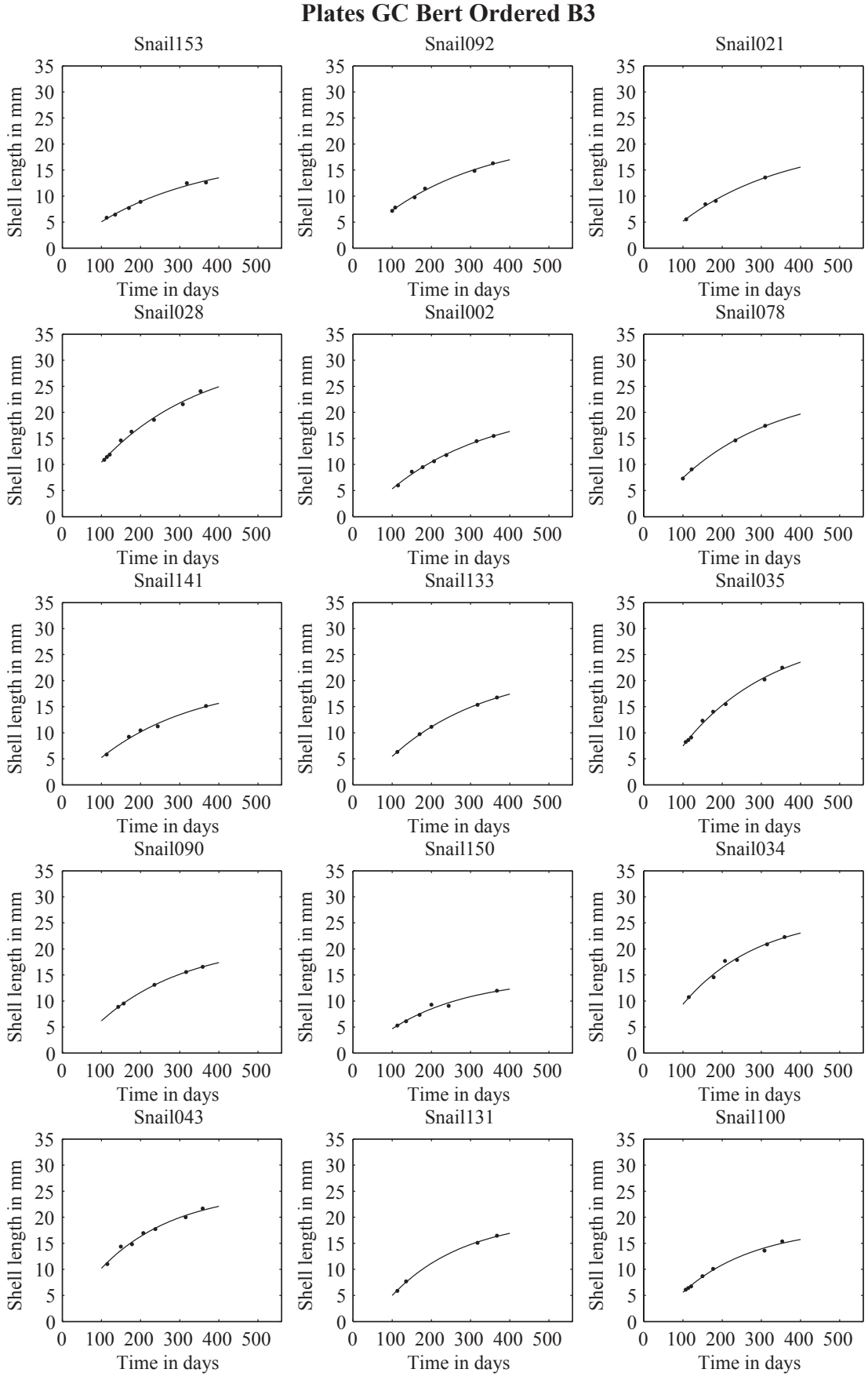

Plates Allo
Snail112
SL:poly3
SW:poly1
Snail052
SL:poly3
SW:poly3
Snail071
SL:poly3
SW:poly3
Snail057
SL:poly3
SW:poly3
Snail143
SL:poly3
SW:poly3
Snail139
SL:poly3
SW:poly3
Snail047
SL:poly3
SW:poly1
Snail148
SL:poly3
SW:poly3
Snail055
SL:poly3
SW:poly3
Snail095
SL:poly3
SW:poly3
Snail116
SL:poly3
SW:poly3
Snail022
SL:poly3
SW:poly3
Snail072
SL:poly3
SW:poly3
Snail044
SL:poly3
SW:poly3
Snail092
SL:poly3
SW:poly3
Shell length in mm
Shell width in mm

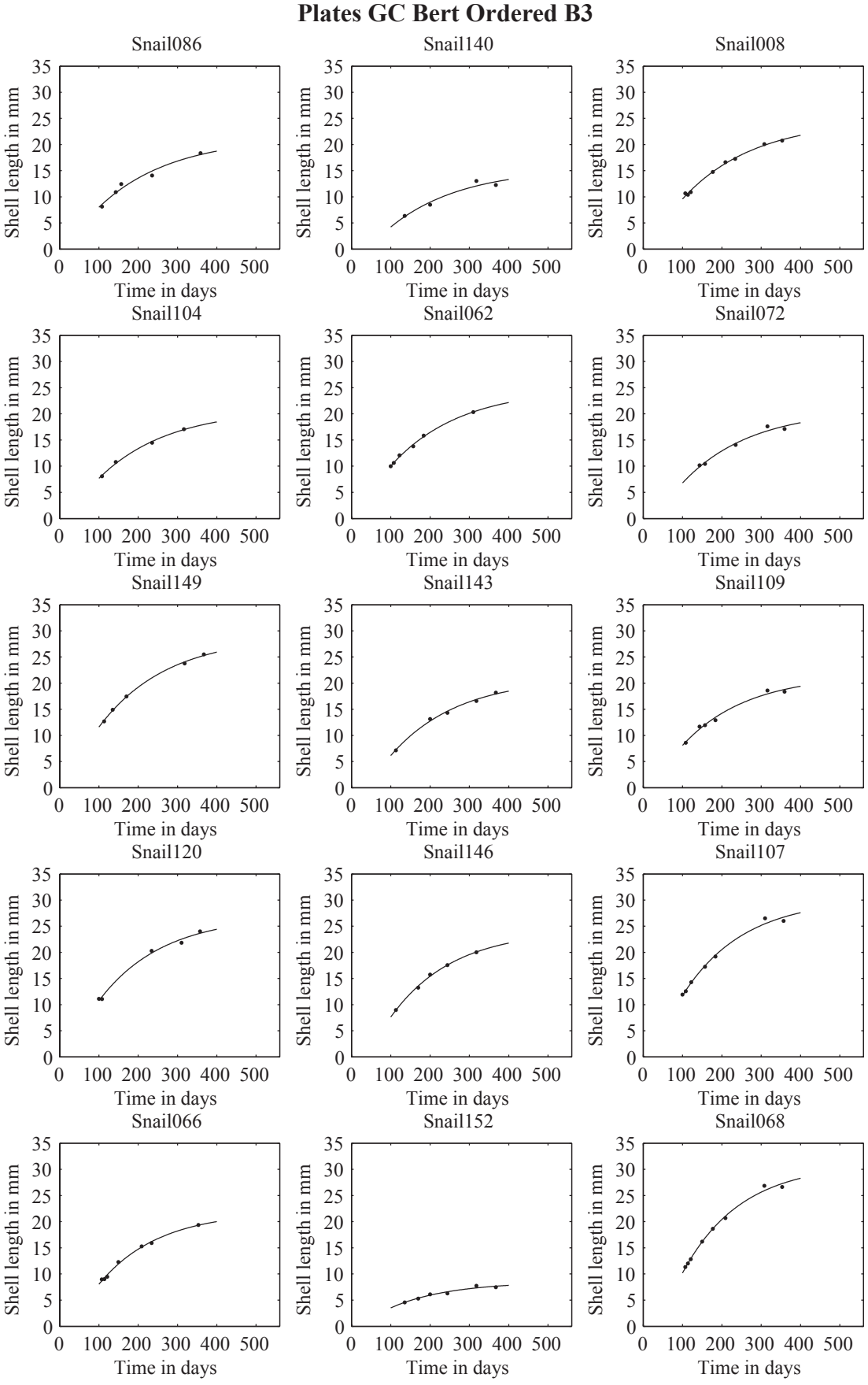

Plates Allo
Snail086
Snail035
Snail078
Snail146
Snail054
Snail098
Snail073
Snail066
Snail109
Snail046
Snail009
Snail010
Snail024
Snail018
Snail082
Shell length in mm
Shell width in mm
SL:poly3
SW:poly3
SW:poly2

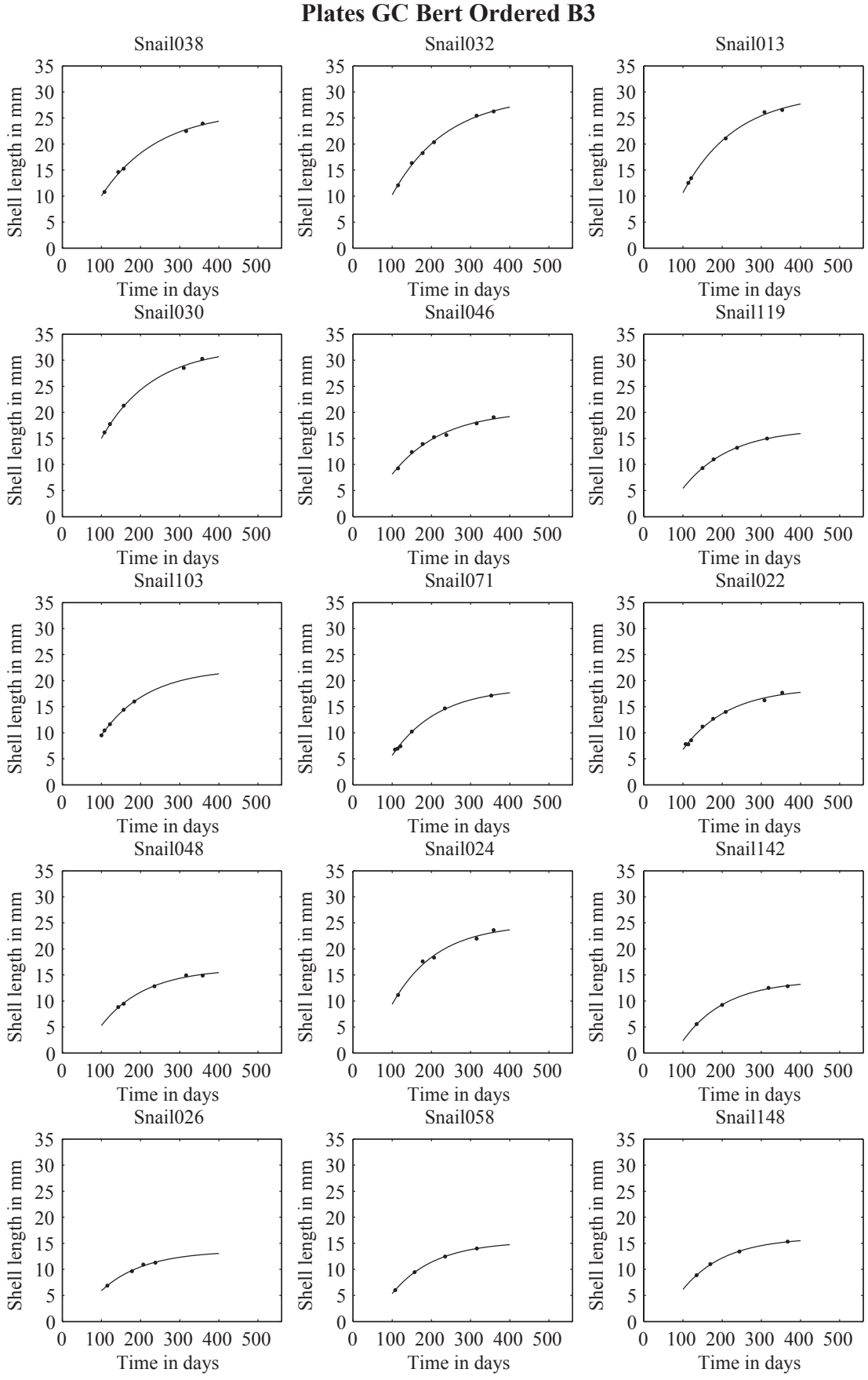

Plates Allo
Snail034
SL:poly3
SW:poly2
Snail028
SL:poly3
SW:poly3
Snail103
SL:poly3
SW:poly2
Snail041
SL:poly3
SW:poly3
Snail043
SL:poly3
SW:poly3
Snail068
SL:poly3
SW:poly3
Snail062
SL:poly3
SW:poly2
Snail038
SL:poly3
SW:poly3
Snail032
SL:poly3
SW:poly3
Snail013
SL:poly3
SW:poly3
Snail120
SL:poly3
SW:poly3
Snail149
SL:poly3
SW:poly3
Snail012
SL:poly3
SW:poly3
Snail107
SL:poly3
SW:poly3
Snail030
SL:poly3
SW:poly3
Shell length in mm
Shell width in mm

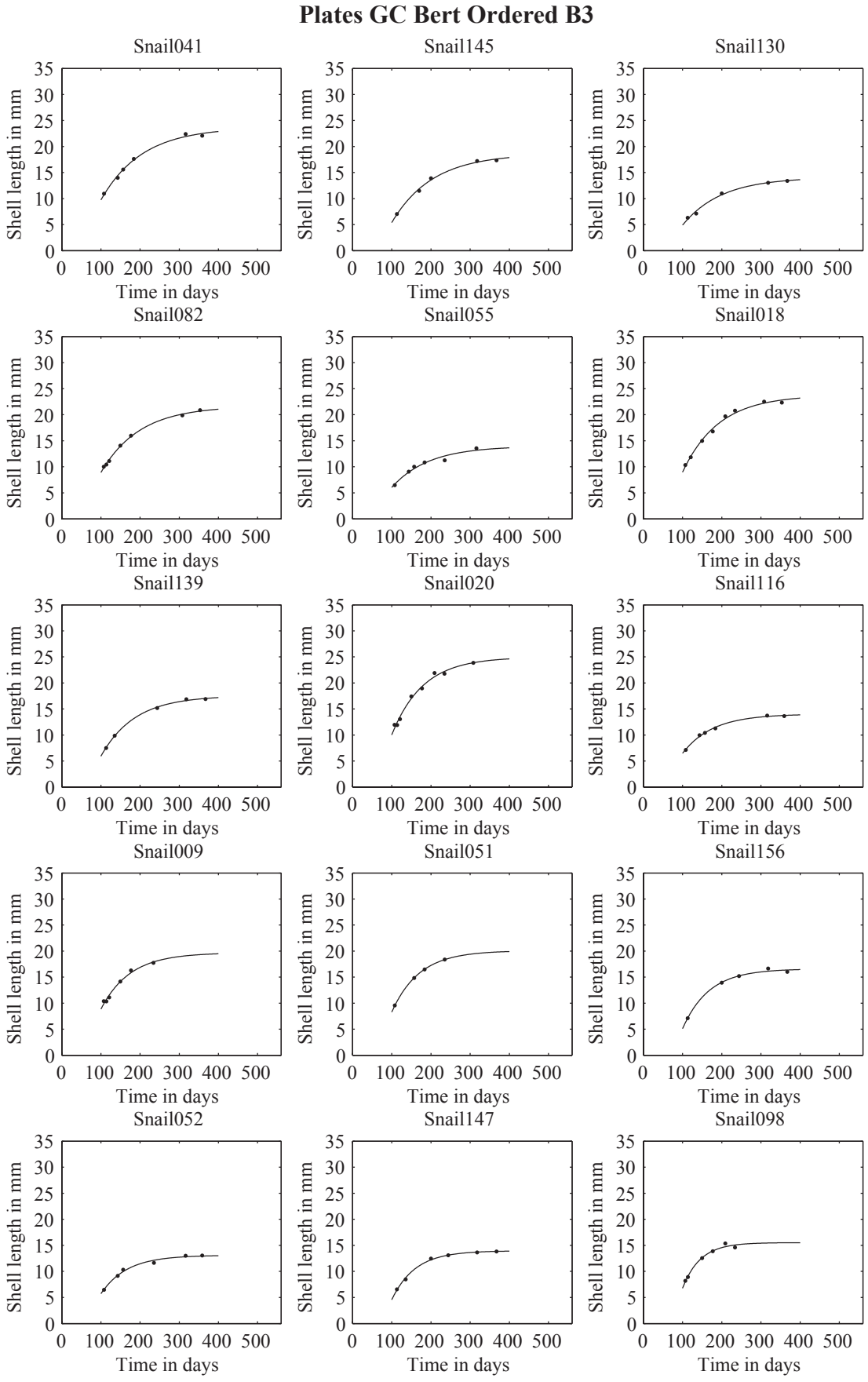

Plates GC Bert Ordered B3
Snail201
Snail056
Snail083
Snail112
Snail031
Snail064
Snail136
Snail067
Snail134
Snail044
Snail029
Snail007
Snail006
Snail023
Snail111
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

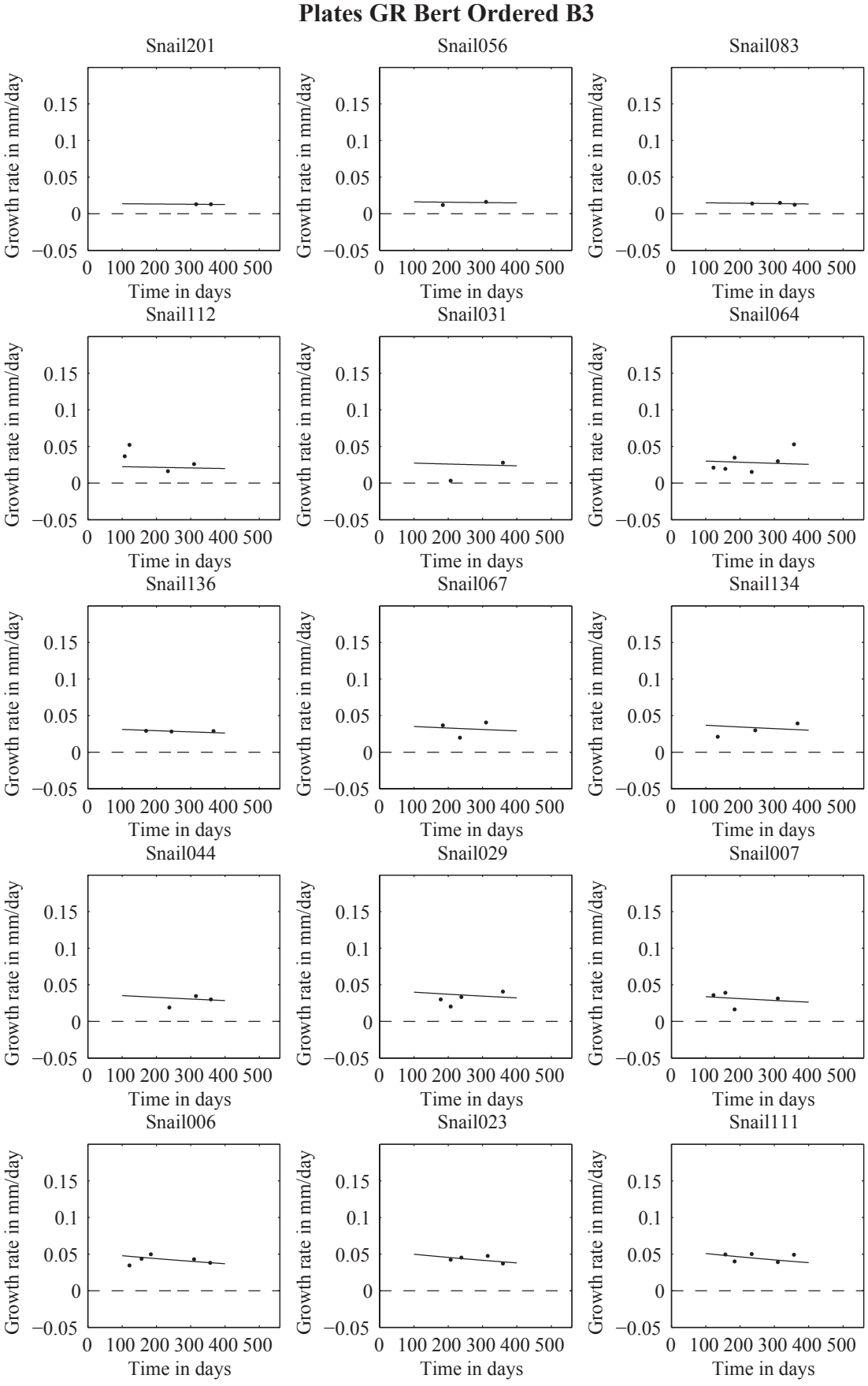

Plates GC Bert Ordered B3
Snail065
Snail047
Snail045
Snail053
Snail057
Snail096
Snail088
Snail010
Snail061
Snail095
Snail036
Snail012
Snail063
Snail073
Snail144
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

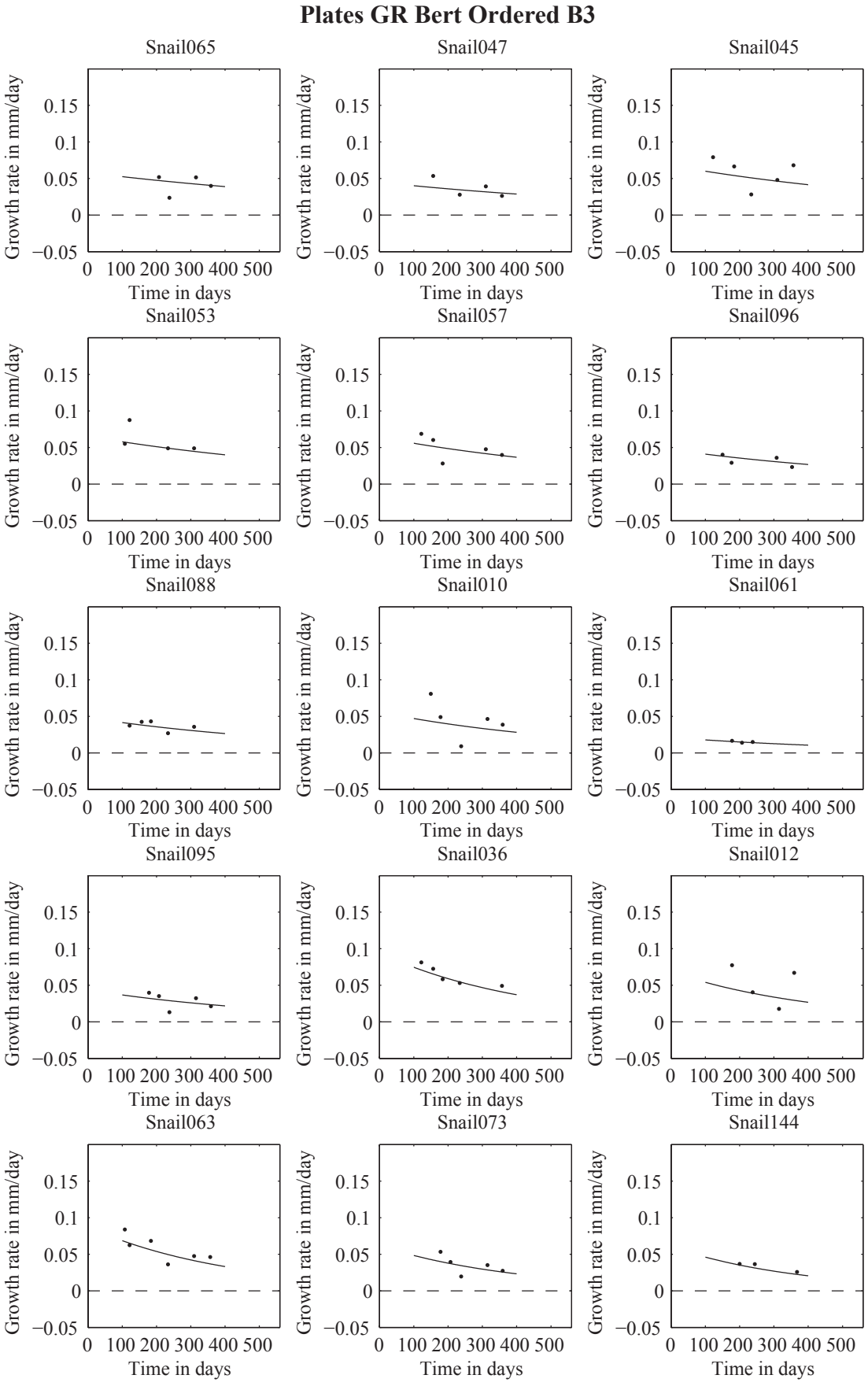

Plates GC Bert Ordered B3
Snail153
Snail092
Snail021
Snail028
Snail002
Snail078
Snail141
Snail133
Snail035
Snail090
Snail150
Snail034
Snail043
Snail131
Snail100
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

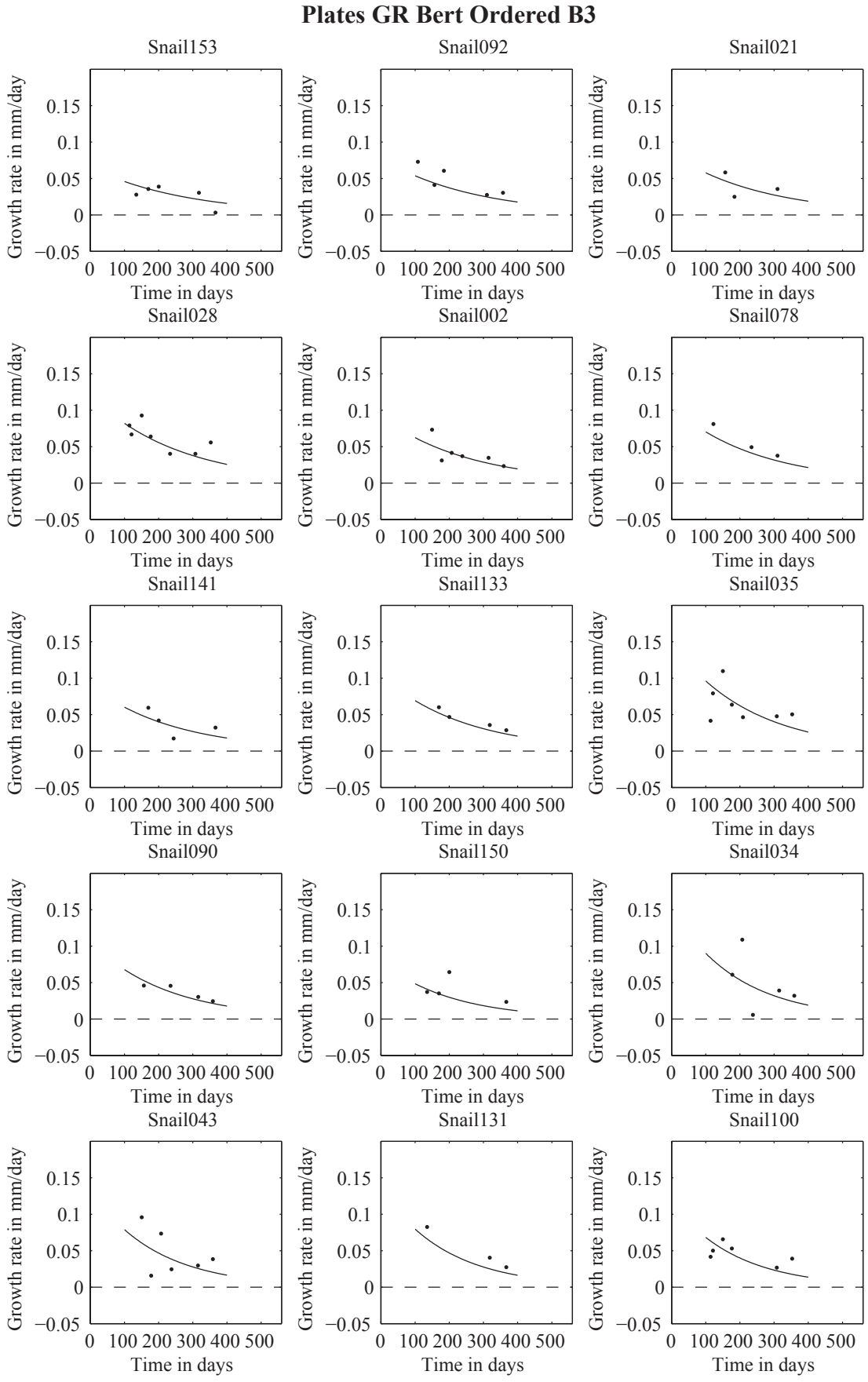

Plates GC Bert Ordered B3
Snail086
Snail140
Snail008
Snail104
Snail062
Snail072
Snail149
Snail143
Snail109
Snail120
Snail146
Snail107
Snail066
Snail152
Snail068
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

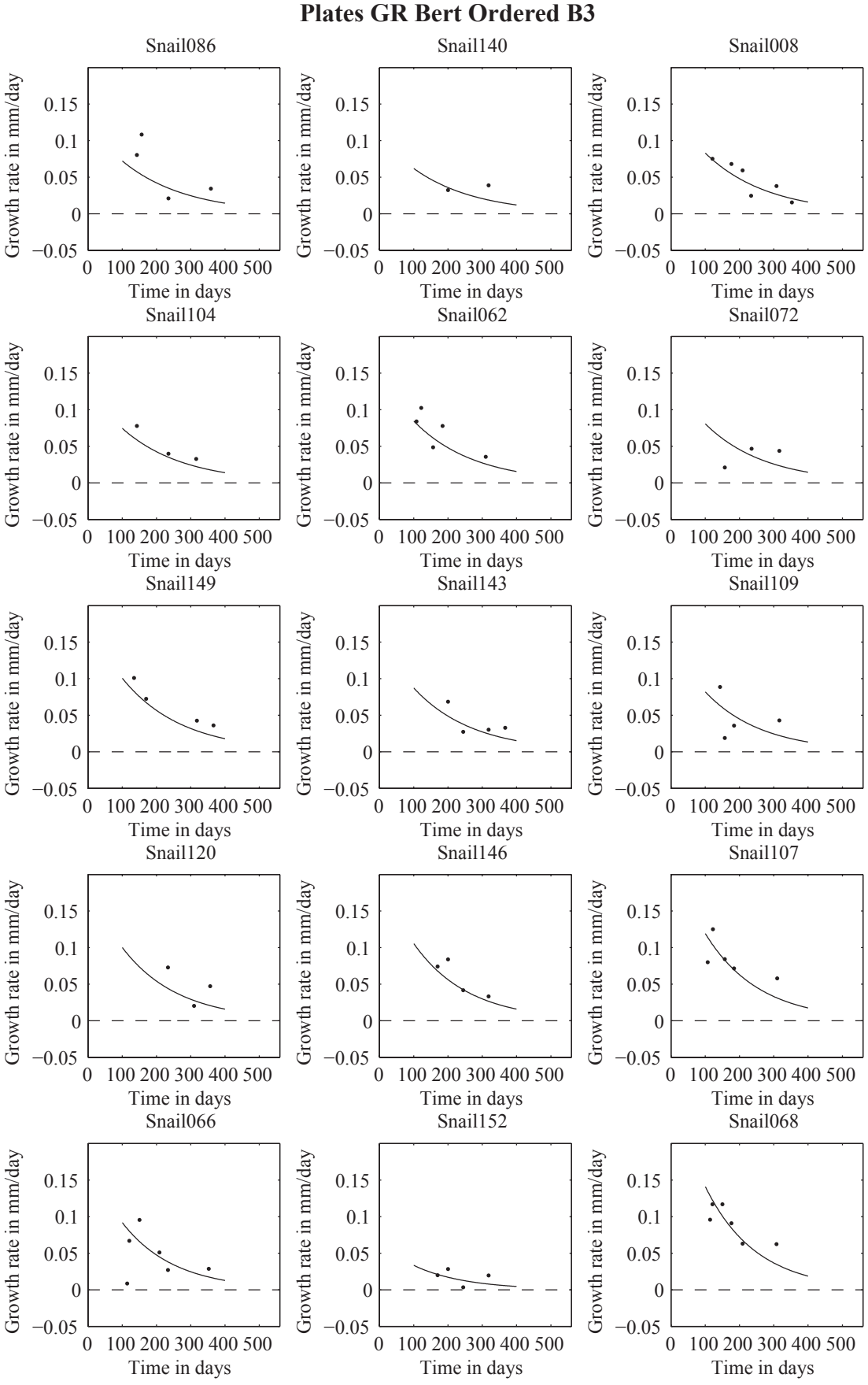

Plates GC Bert Ordered B3
Snail038
Snail032
Snail013
Snail030
Snail046
Snail119
Snail103
Snail071
Snail022
Snail048
Snail024
Snail142
Snail026
Snail058
Snail148
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

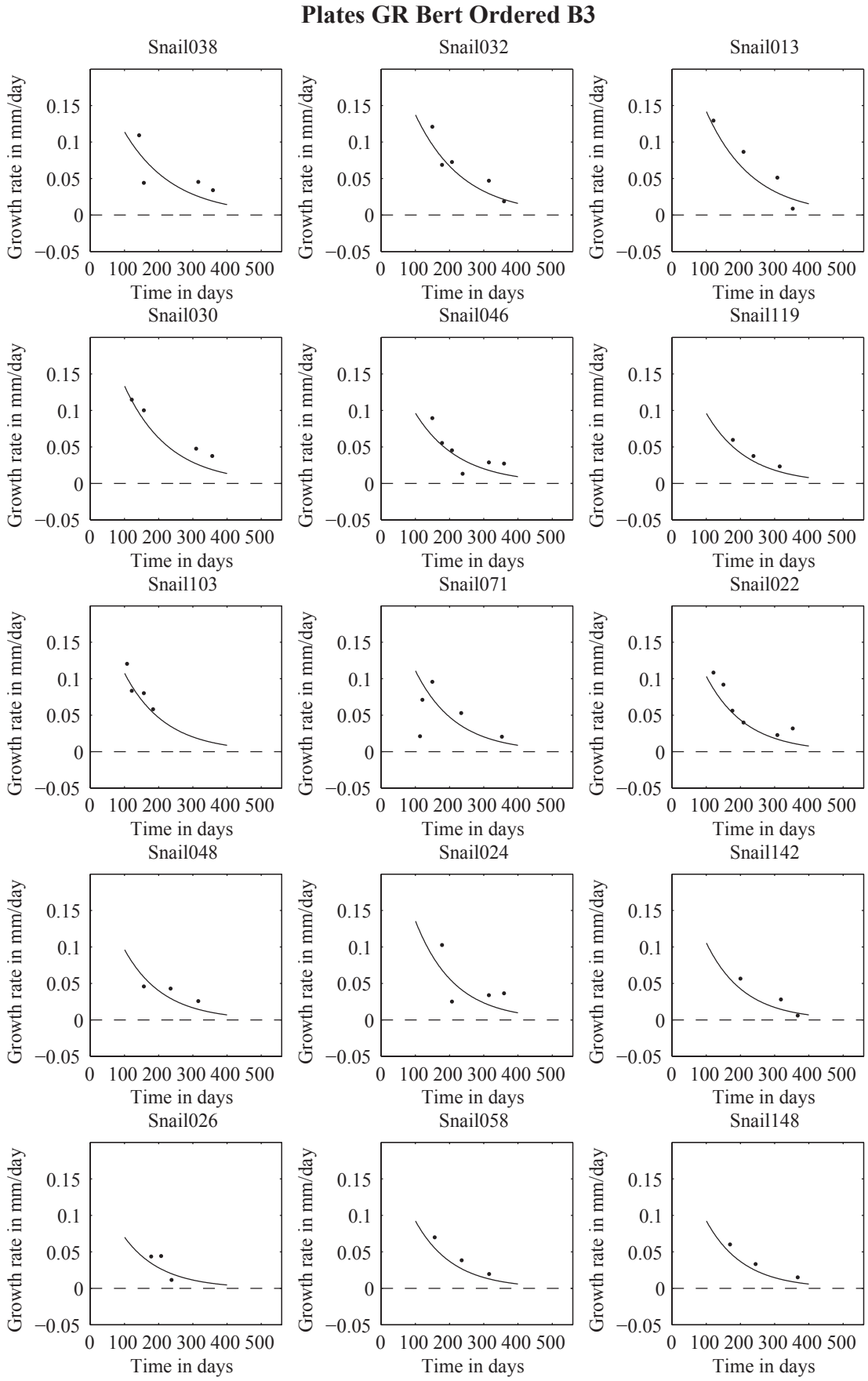

Plates GC Bert Ordered B3
Snail041
Snail145
Snail130
Snail082
Snail055
Snail018
Snail139
Snail020
Snail116
Snail009
Snail051
Snail156
Snail052
Snail147
Snail098
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

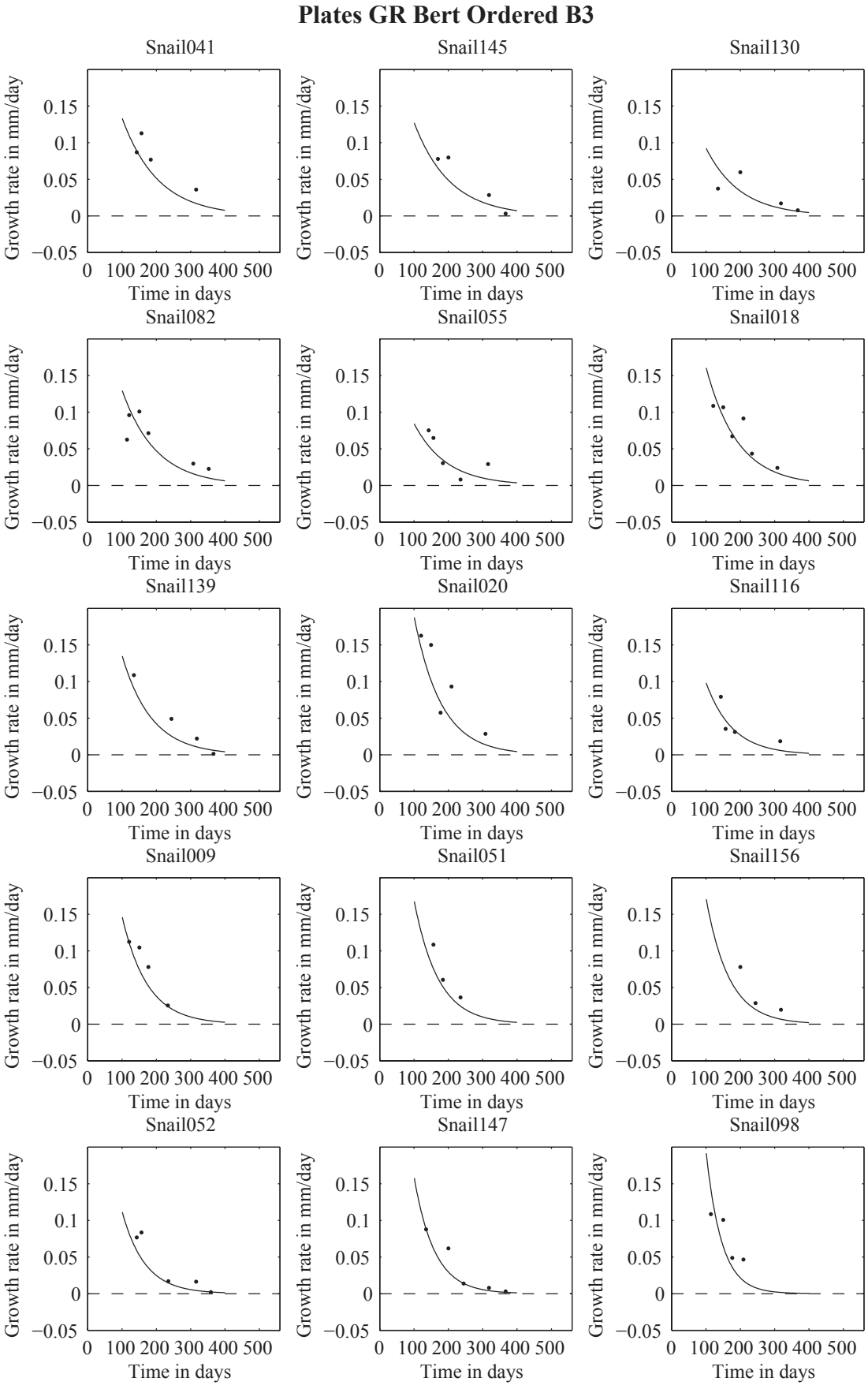

Plates GR Bert Ordered B3
Snail201
Snail056
Snail083
Snail112
Snail031
Snail064
Snail136
Snail067
Snail134
Snail044
Snail029
Snail007
Snail006
Snail023
Snail111
Growth rate in mm/day
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500
Time in days

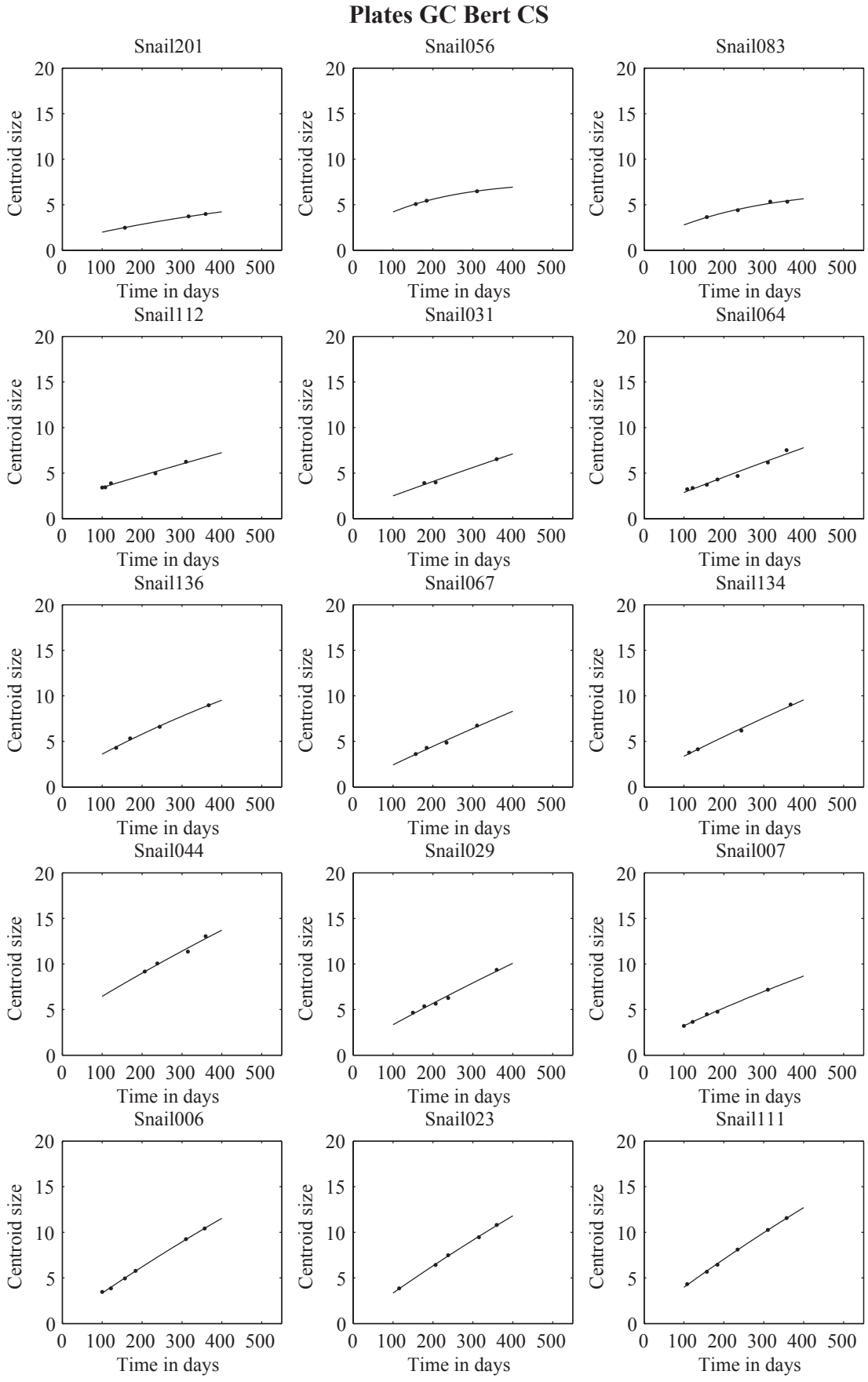

Plates GR Bert Ordered B3
Snail065
Snail047
Snail045
Snail053
Snail057
Snail096
Snail088
Snail010
Snail061
Snail095
Snail036
Snail012
Snail063
Snail073
Snail144
Growth rate in mm/day
Time in days
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500

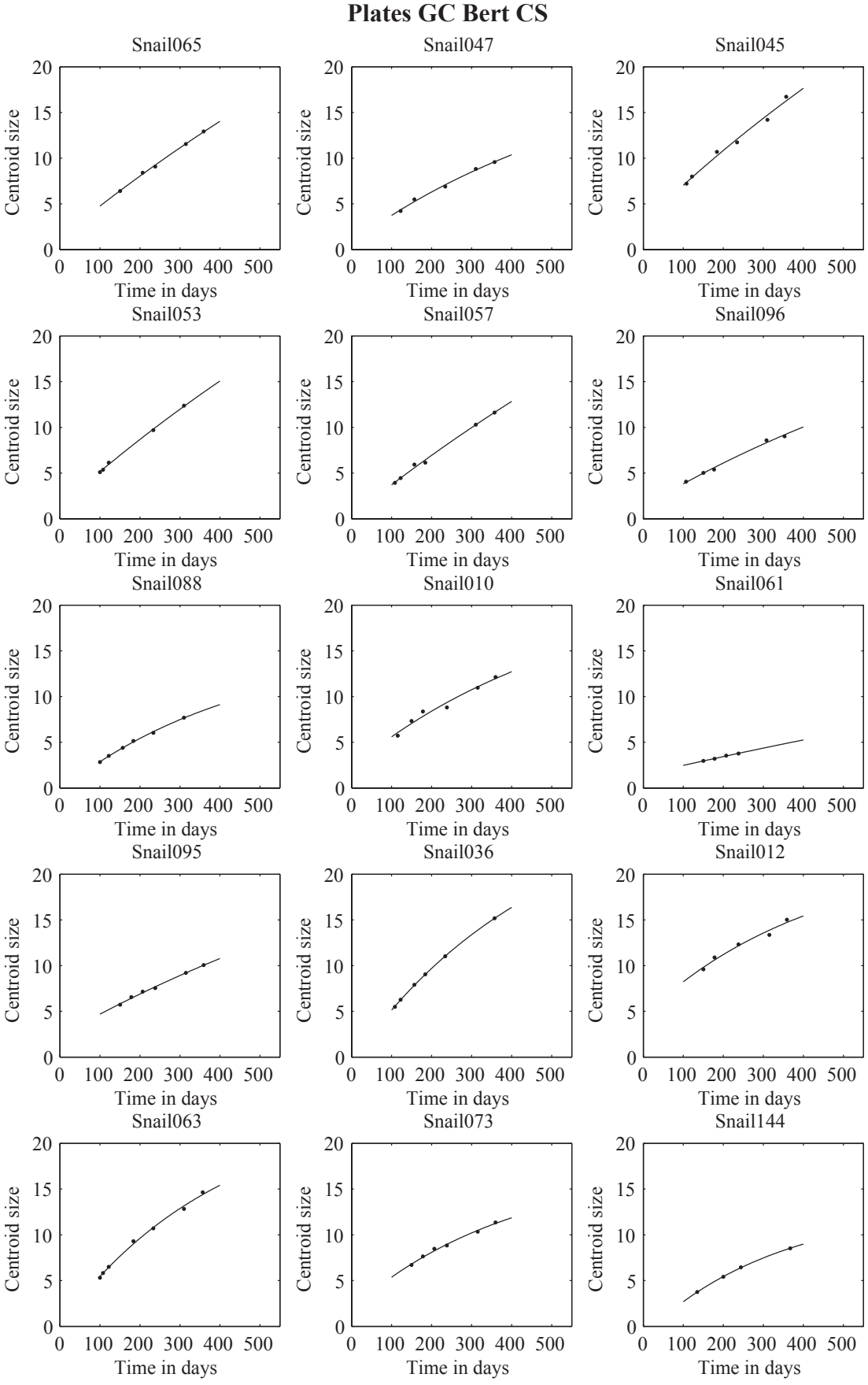

Plates GR Bert Ordered B3
Snail153
Snail092
Snail021
Snail028
Snail002
Snail078
Snail141
Snail133
Snail035
Snail090
Snail150
Snail034
Snail043
Snail131
Snail100
Growth rate in mm/day
Time in days
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500

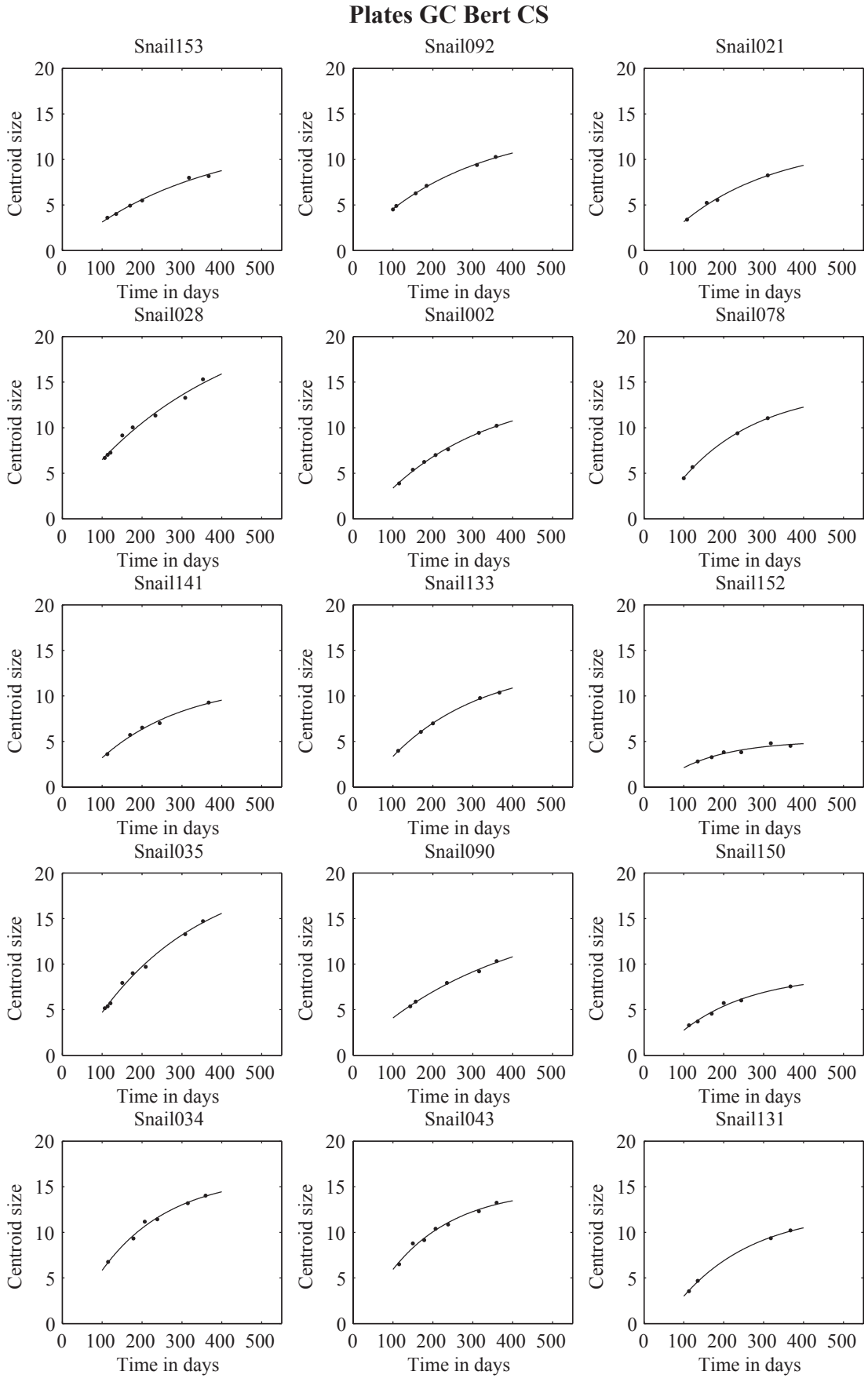

Plates GR Bert Ordered B3
Snail086
Snail140
Snail008
Snail104
Snail062
Snail072
Snail149
Snail143
Snail109
Snail120
Snail146
Snail107
Snail066
Snail152
Snail068
Growth rate in mm/day
Time in days
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500

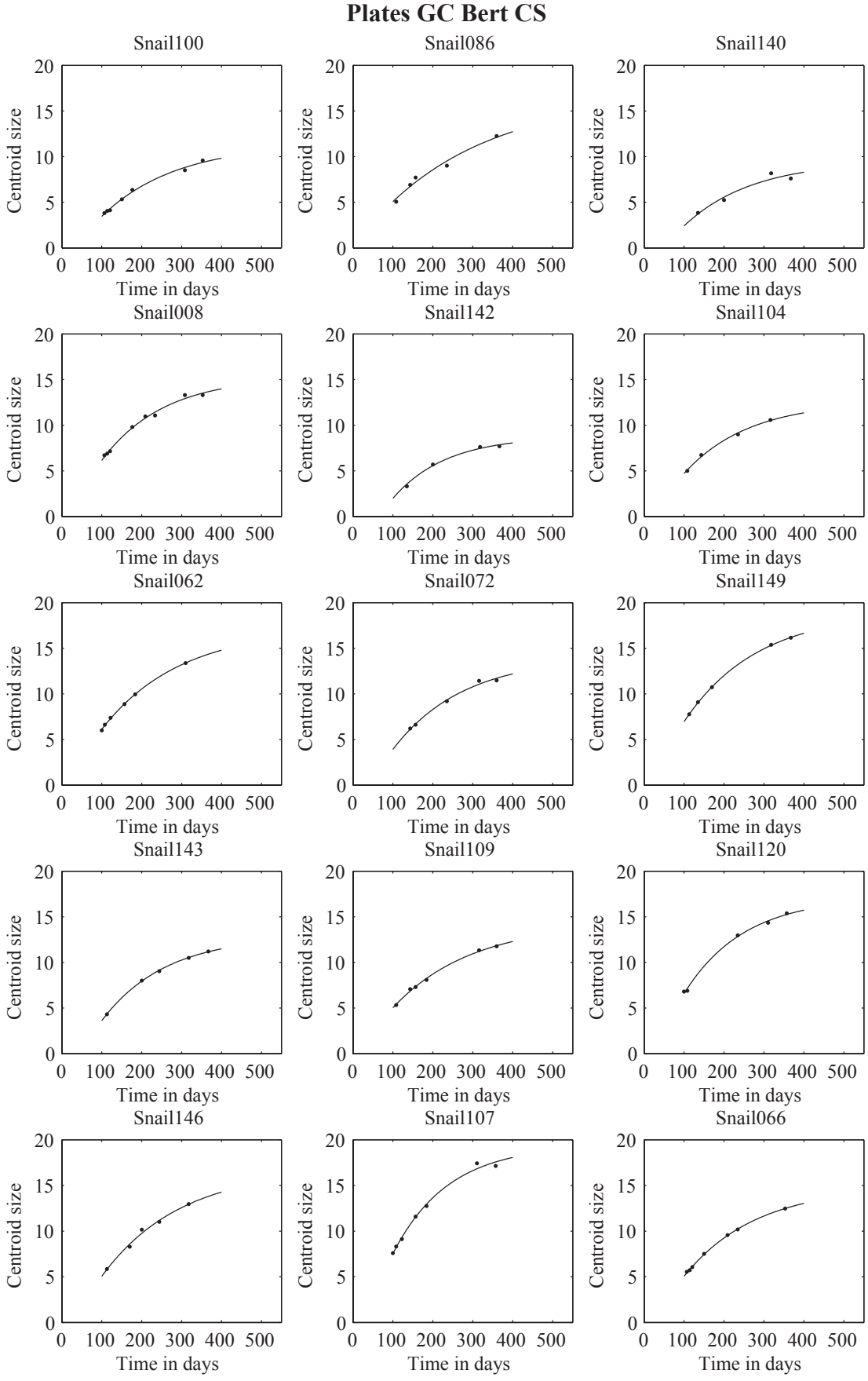

Plates GR Bert Ordered B3
Snail038
Snail032
Snail013
Snail030
Snail046
Snail119
Snail103
Snail071
Snail022
Snail048
Snail024
Snail142
Snail026
Snail058
Snail148
Growth rate in mm/day
Time in days
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500

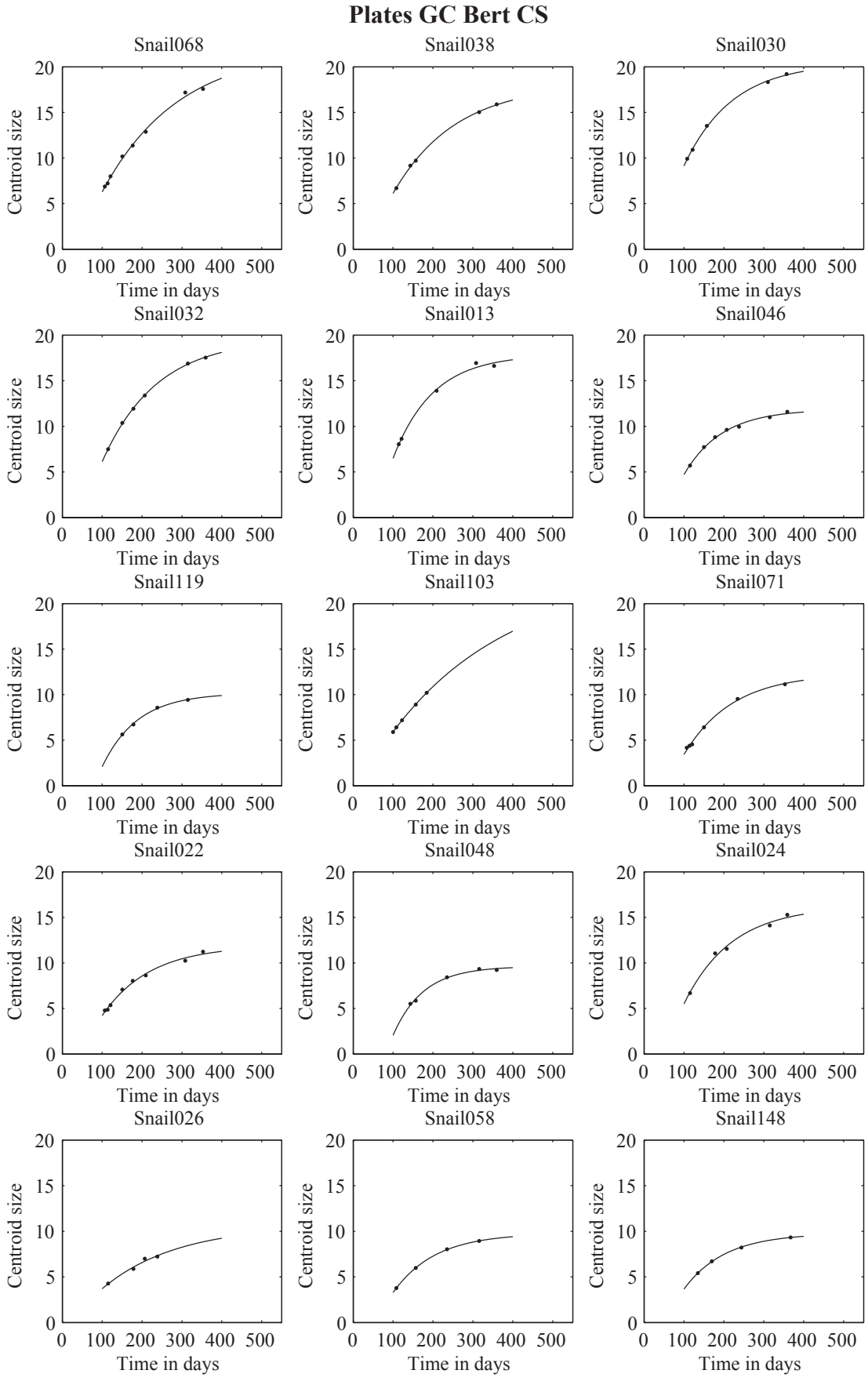

Plates GR Bert Ordered B3
Snail041
Snail145
Snail130
Snail082
Snail055
Snail018
Snail139
Snail020
Snail116
Snail009
Snail051
Snail156
Snail052
Snail147
Snail098
Growth rate in mm/day
Time in days
0.15
0.1
0.05
0
−0.05
0 100 200 300 400 500

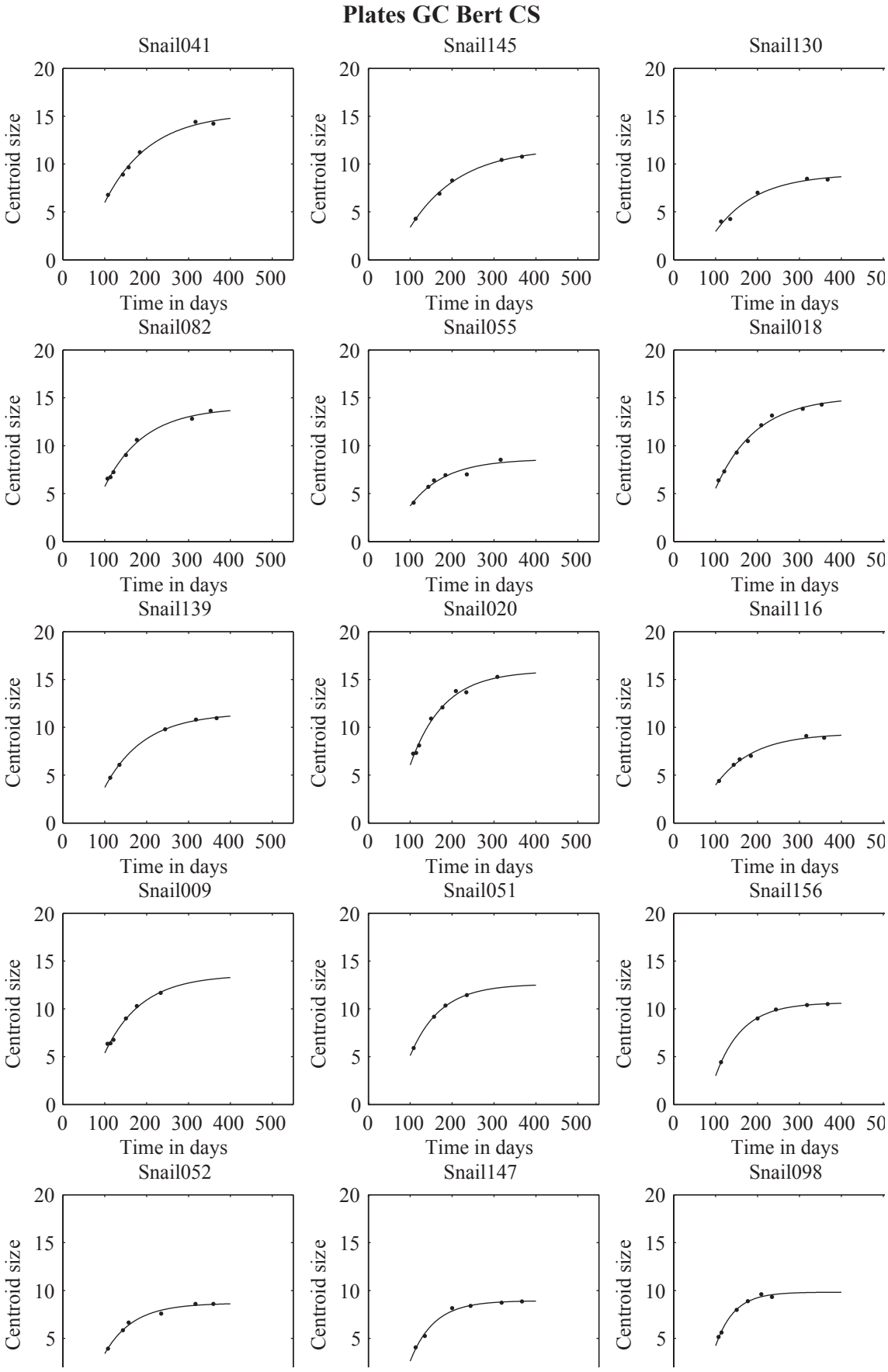

Plates GC Bert CS
Snail201
Snail056
Snail083
Snail112
Snail031
Snail064
Snail136
Snail067
Snail134
Snail044
Snail029
Snail007
Snail006
Snail023
Snail111
Centroid size
Time in days
0
5
10
15
20
0 100 200 300 400 500

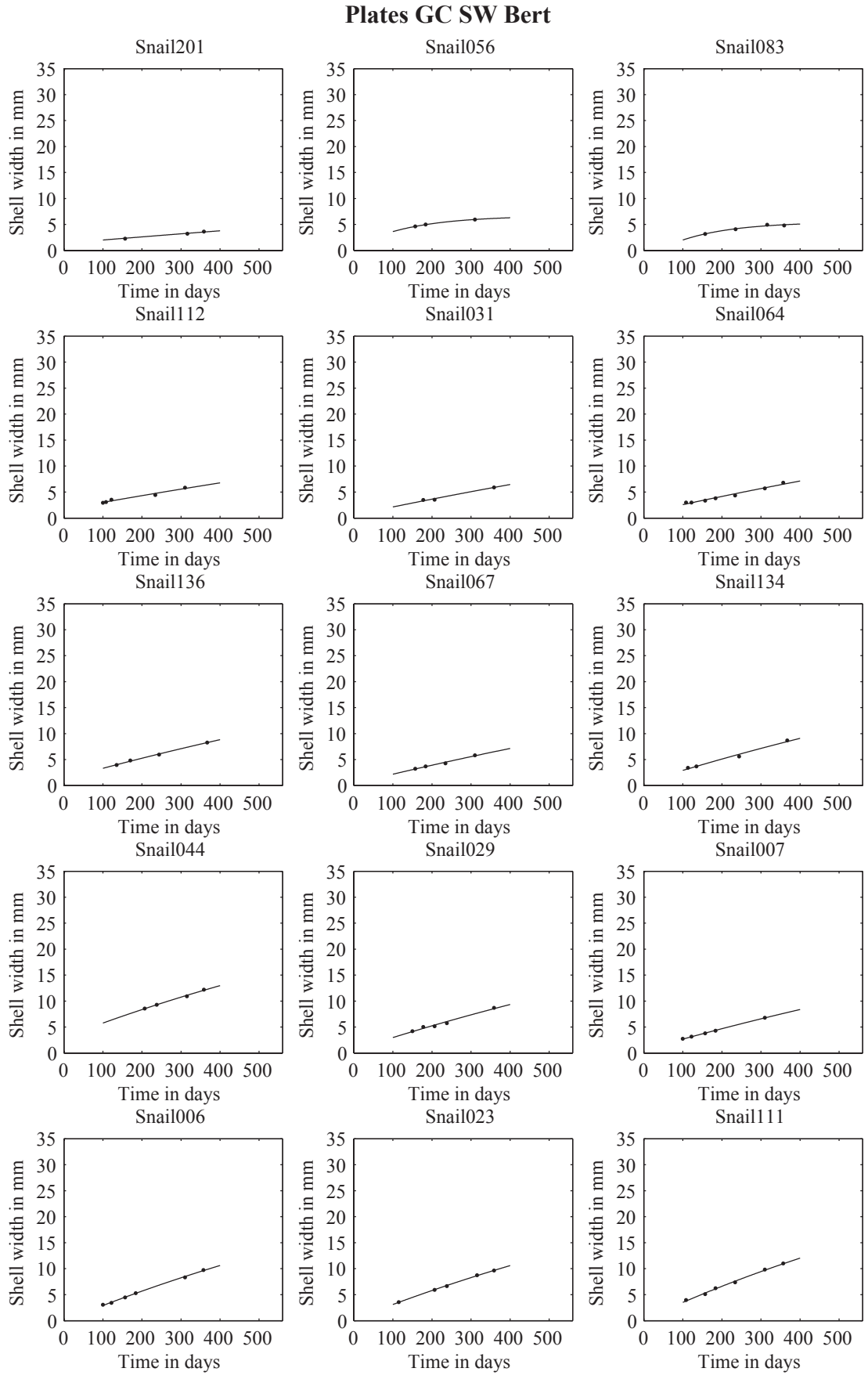

Plates GC Bert CS
Snail065
Snail047
Snail045
Snail053
Snail057
Snail096
Snail088
Snail010
Snail061
Snail095
Snail036
Snail012
Snail063
Snail073
Snail144
Centroid size
Time in days
0
5
10
15
20
0 100 200 300 400 500

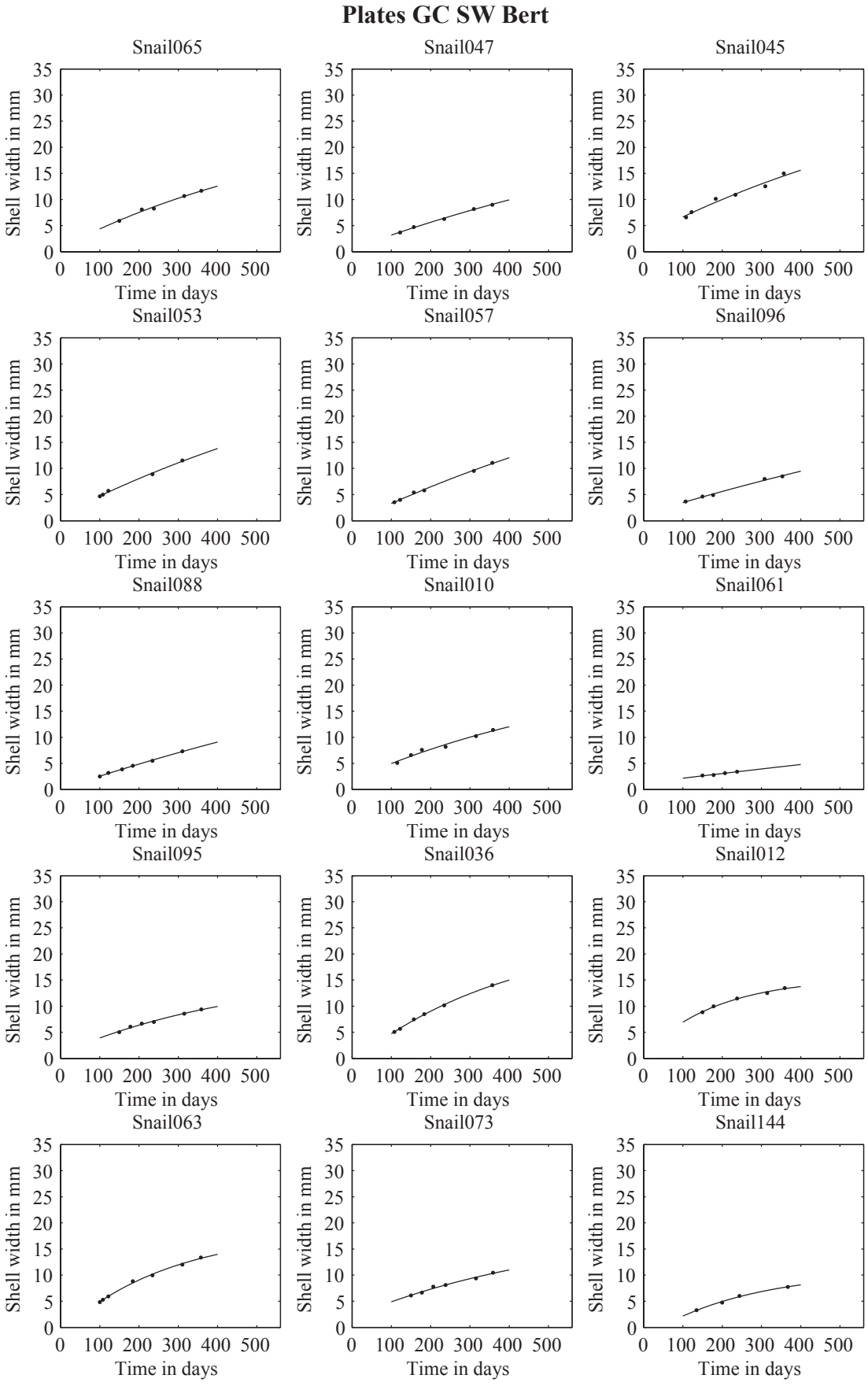

Plates GC Bert CS
Snail153
Snail092
Snail021
Snail028
Snail002
Snail078
Snail141
Snail133
Snail152
Snail035
Snail090
Snail150
Snail034
Snail043
Snail131
Centroid size
Time in days
0
5
10
15
20
0 100 200 300 400 500

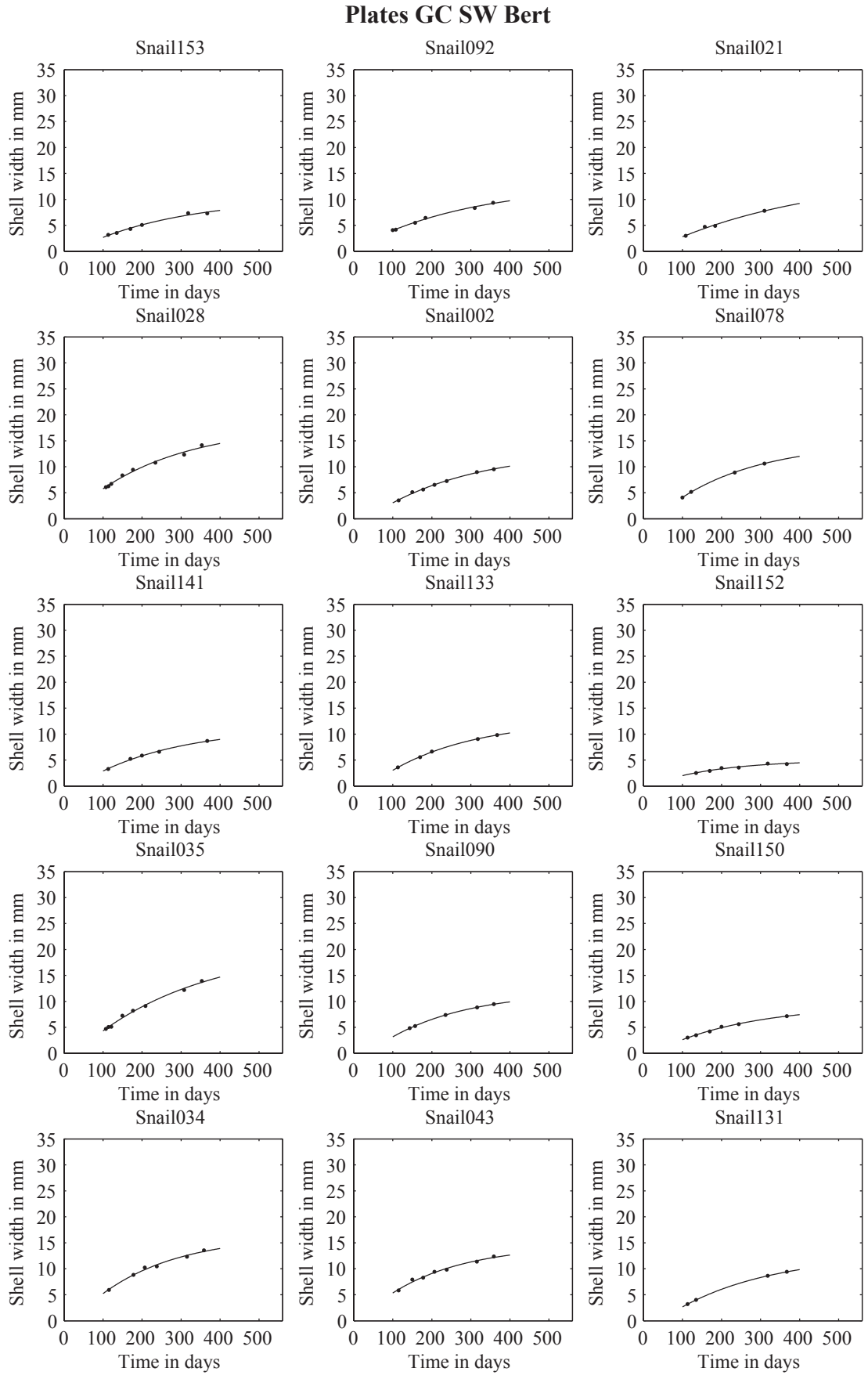

Plates GC Bert CS
Snail100
Snail086
Snail140
Snail008
Snail142
Snail104
Snail062
Snail072
Snail149
Snail143
Snail109
Snail120
Snail146
Snail107
Snail066
Centroid size
Time in days
0 5 10 15 20
0 100 200 300 400 500

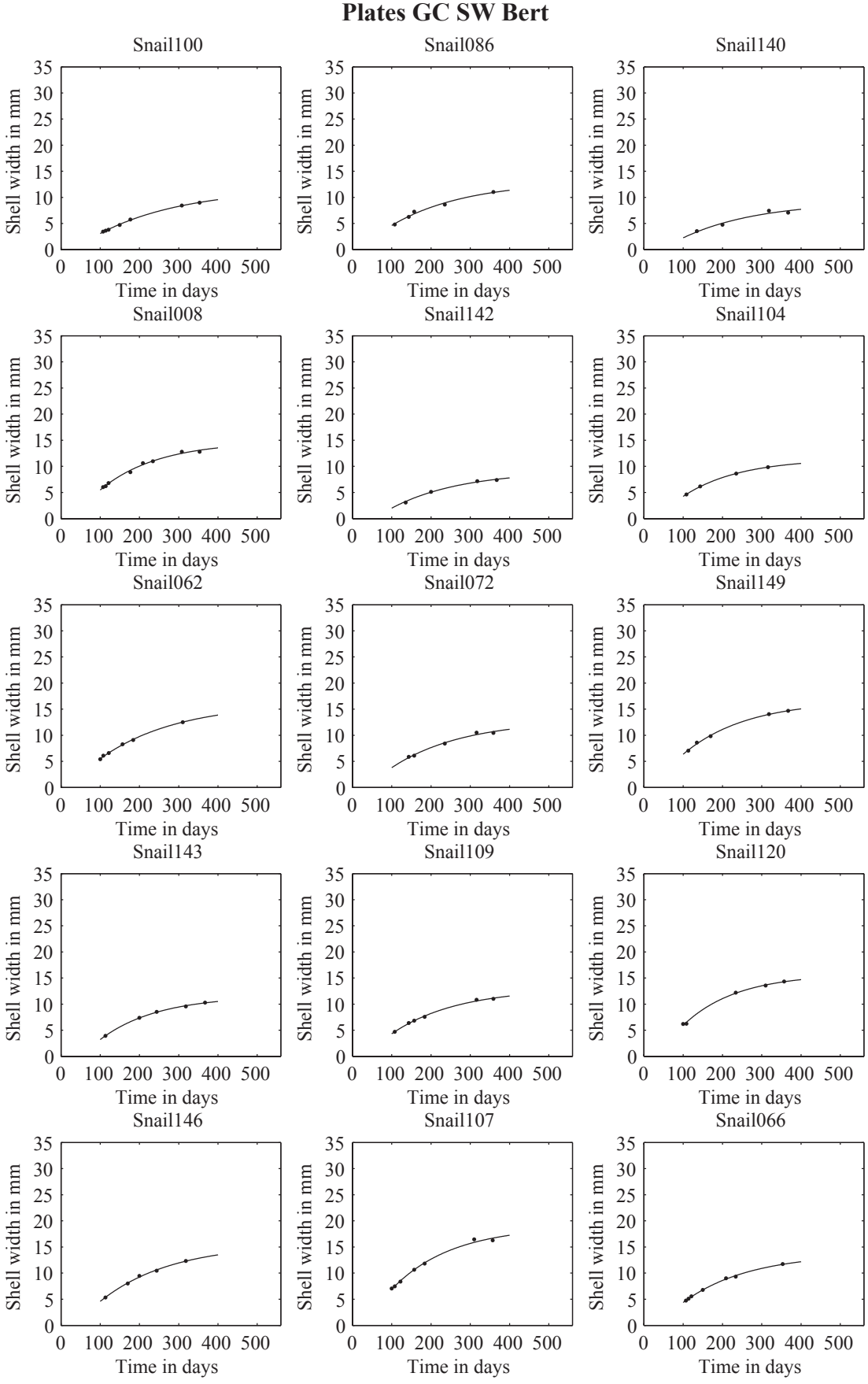

Plates GC Bert CS
Snail068
Snail038
Snail030
Snail032
Snail013
Snail046
Snail119
Snail103
Snail071
Snail022
Snail048
Snail024
Snail026
Snail058
Snail148
Centroid size
Time in days
0
5
10
15
20
0 100 200 300 400 500

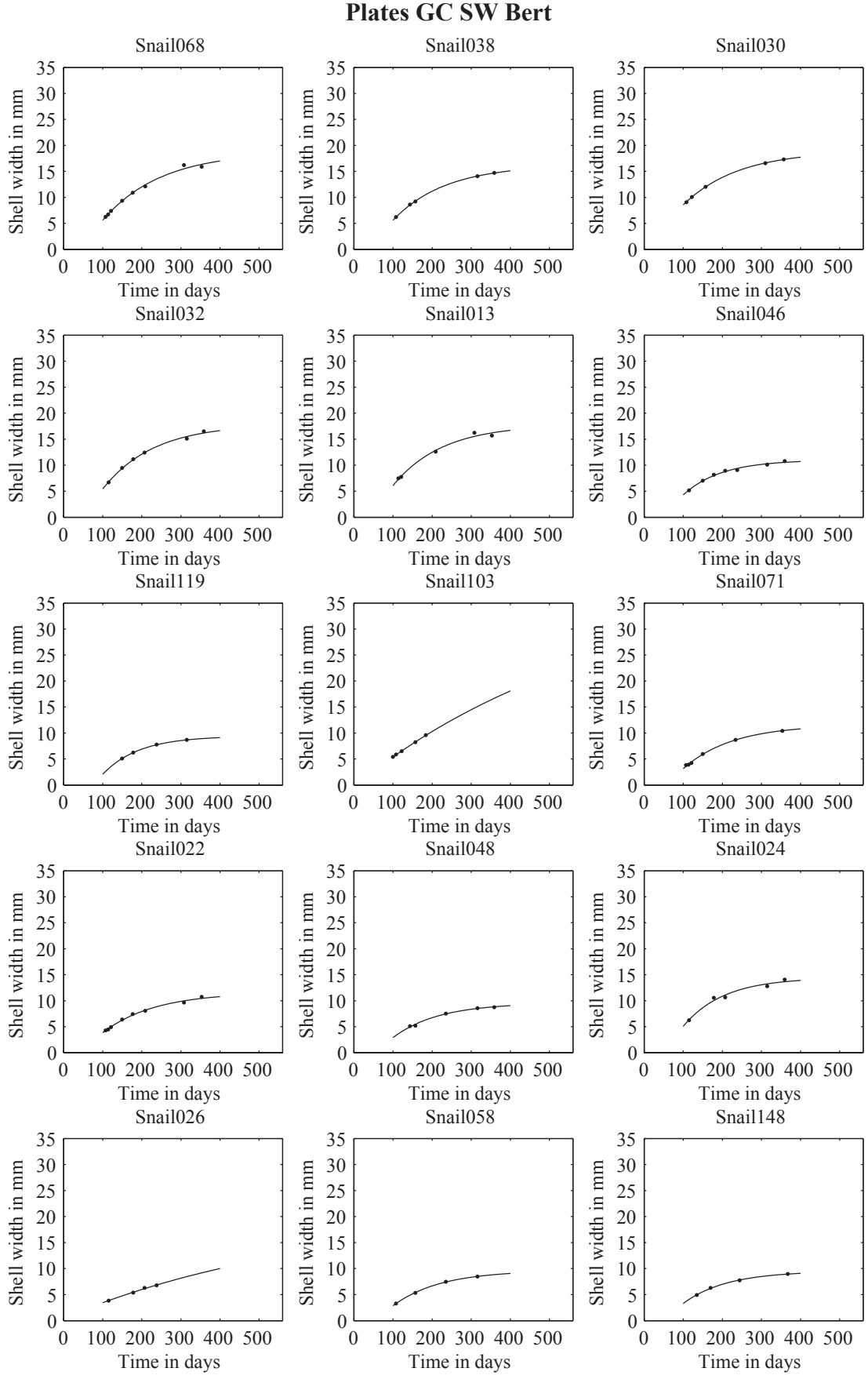

Plates GC Bert CS
Snail041
Snail145
Snail130
Snail082
Snail055
Snail018
Snail139
Snail020
Snail116
Snail009
Snail051
Snail156
Snail052
Snail147
Snail098
Centroid size
Time in days
0
5
10
15
20
0 100 200 300 400 500

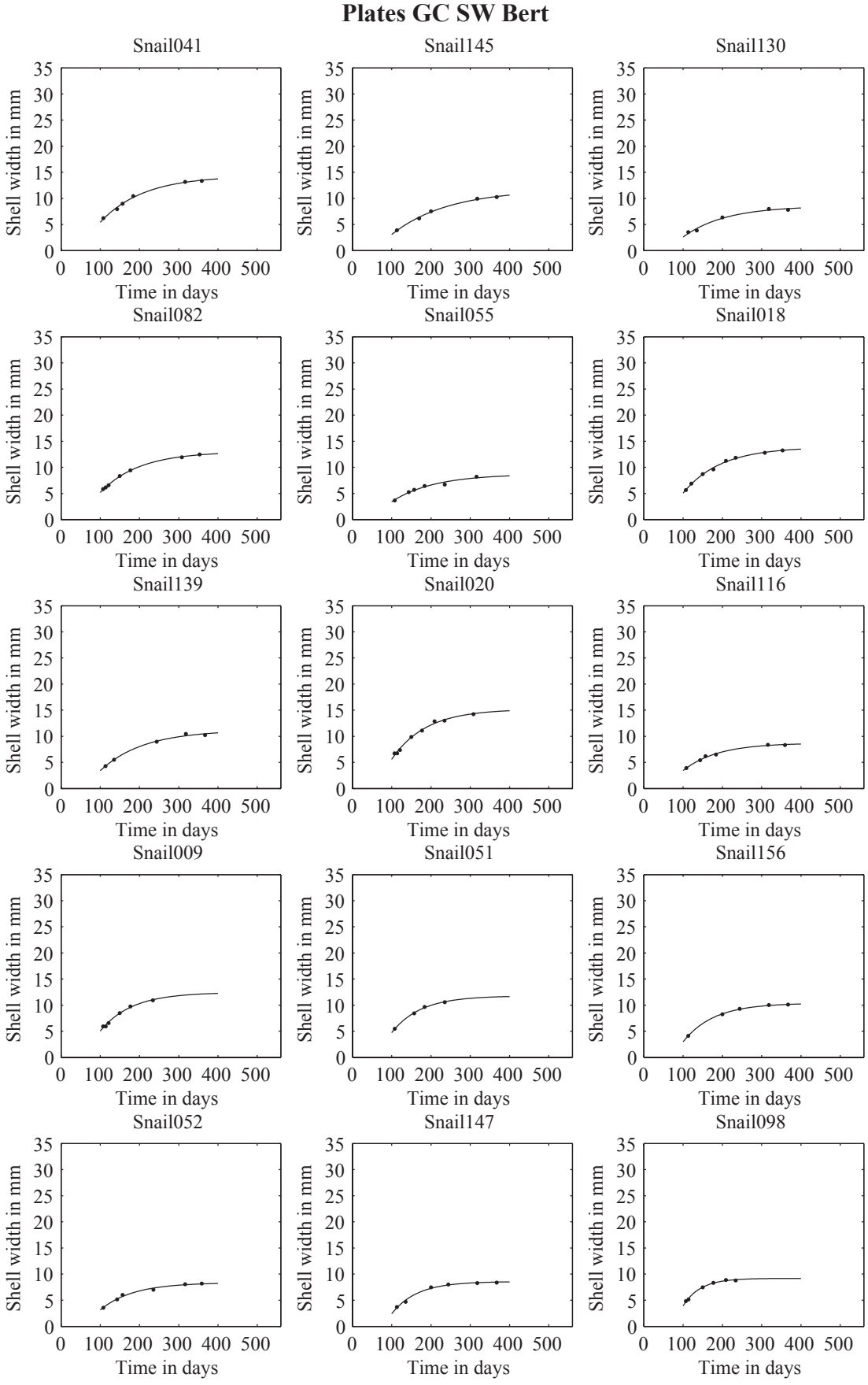

Plates GC SW Bert
Snail201
Snail056
Snail083
Snail112
Snail031
Snail064
Snail136
Snail067
Snail134
Snail044
Snail029
Snail007
Snail006
Snail023
Snail111
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

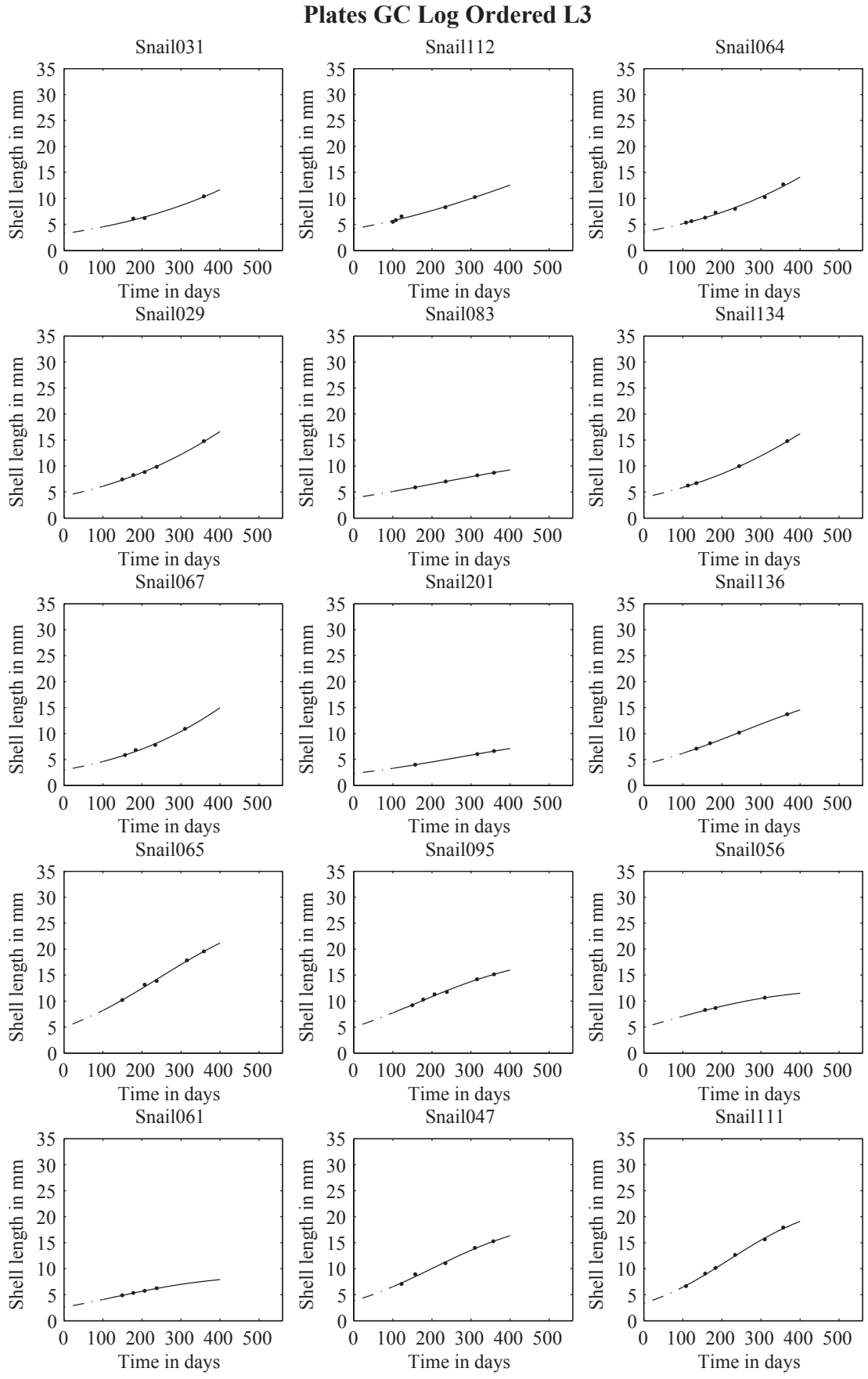

Plates GC SW Bert
Snail065
Snail047
Snail045
Snail053
Snail057
Snail096
Snail088
Snail010
Snail061
Snail095
Snail036
Snail012
Snail063
Snail073
Snail144
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

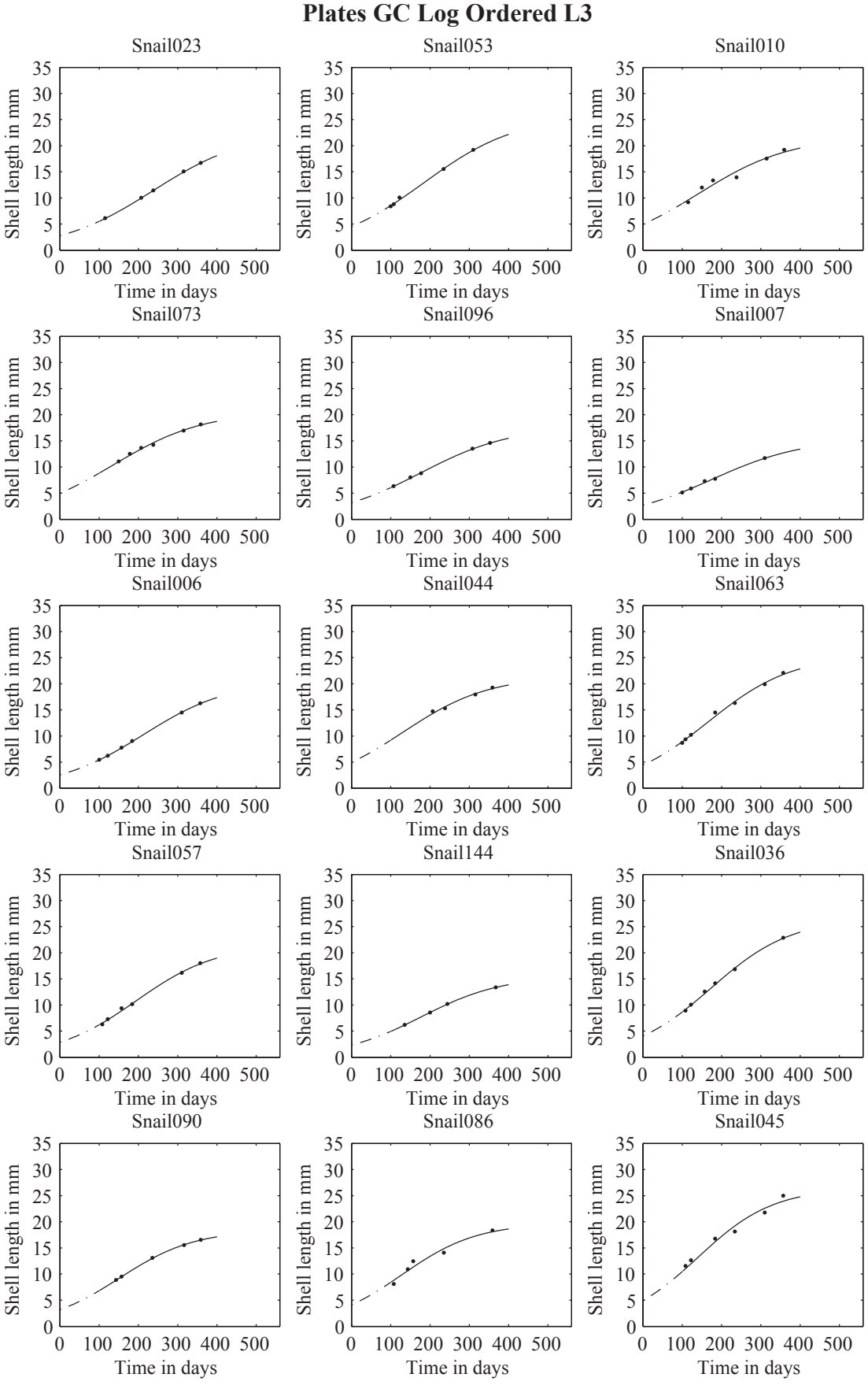

Plates GC SW Bert
Snail153
Snail092
Snail021
Snail028
Snail002
Snail078
Snail141
Snail133
Snail152
Snail035
Snail090
Snail150
Snail034
Snail043
Snail131
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

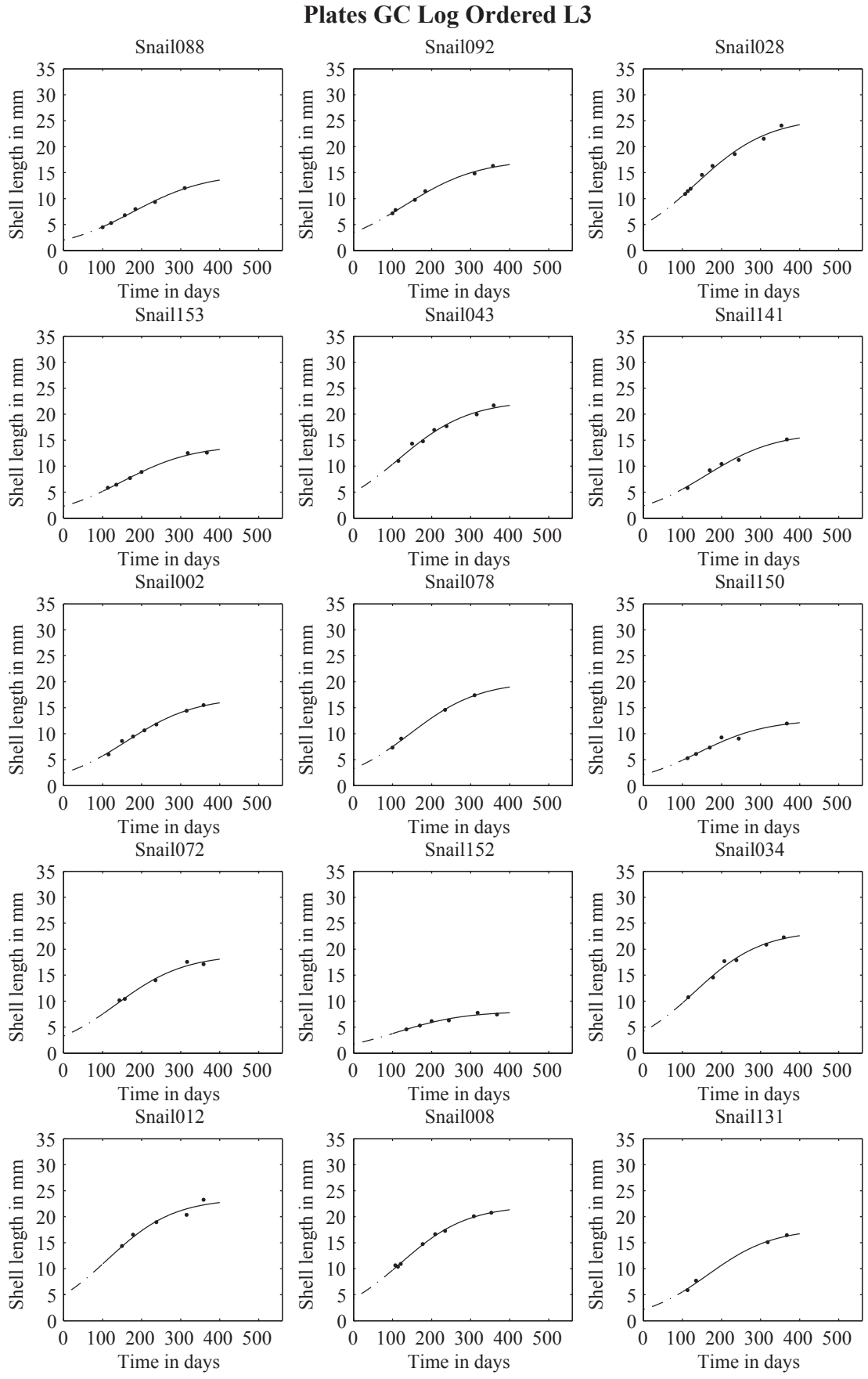

Plates GC SW Bert
Snail100
Snail086
Snail140
Snail008
Snail142
Snail104
Snail062
Snail072
Snail149
Snail143
Snail109
Snail120
Snail146
Snail107
Snail066
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

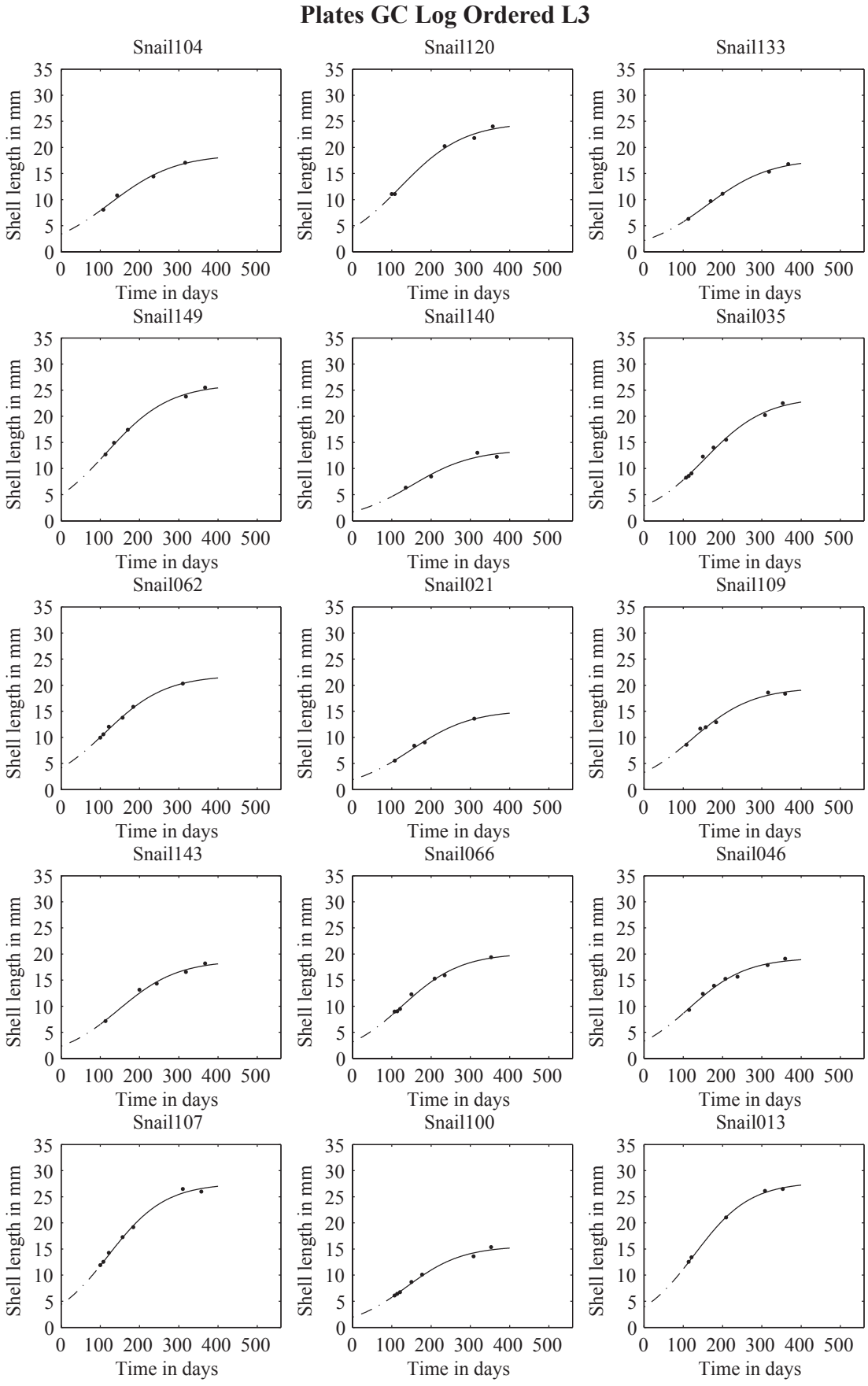

Plates GC SW Bert
Snail068
Snail038
Snail030
Snail032
Snail013
Snail046
Snail119
Snail103
Snail071
Snail022
Snail048
Snail024
Snail026
Snail058
Snail148
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

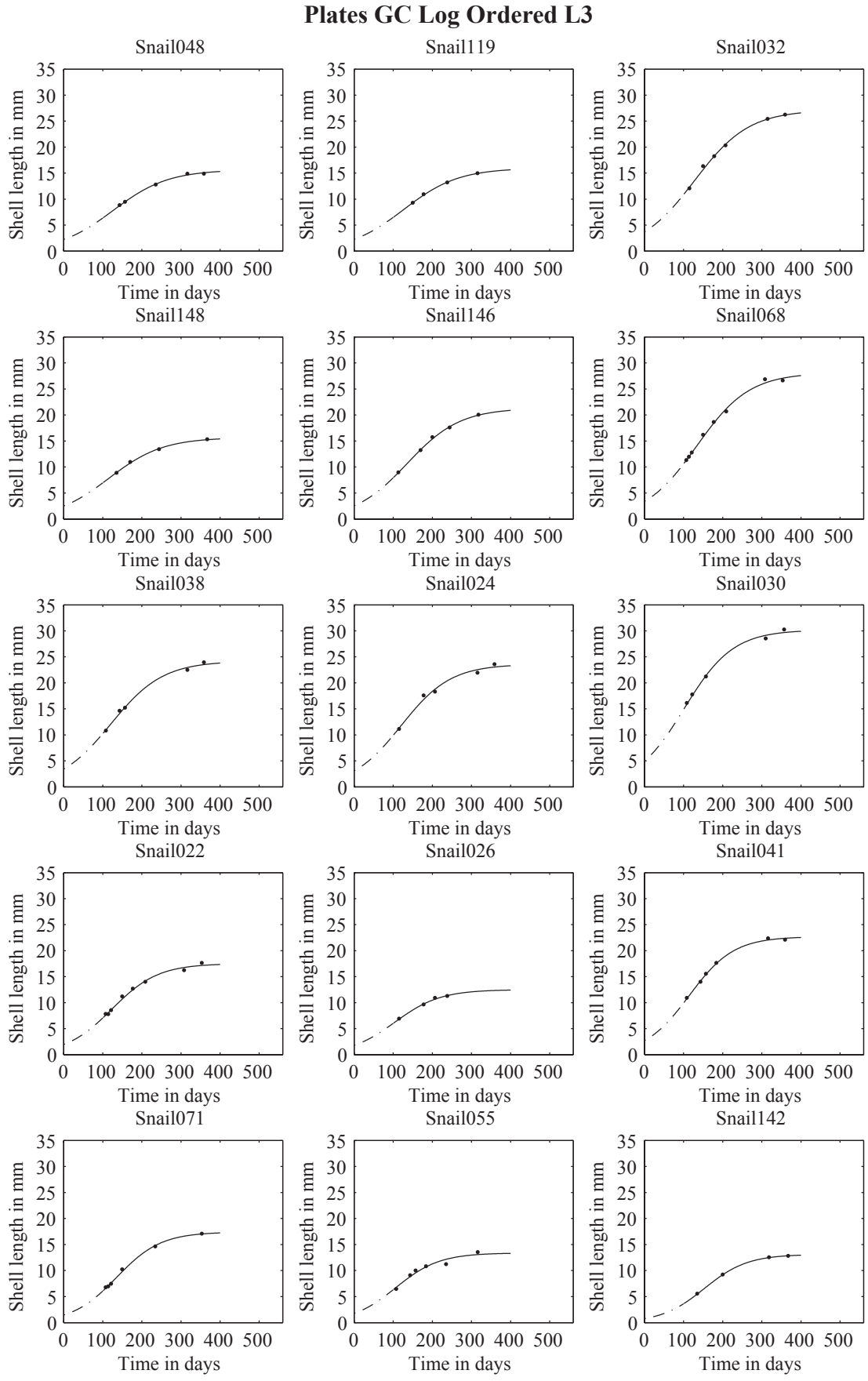

Plates GC SW Bert
Snail041
Snail145
Snail130
Snail082
Snail055
Snail018
Snail139
Snail020
Snail116
Snail009
Snail051
Snail156
Snail052
Snail147
Snail098
Shell width in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

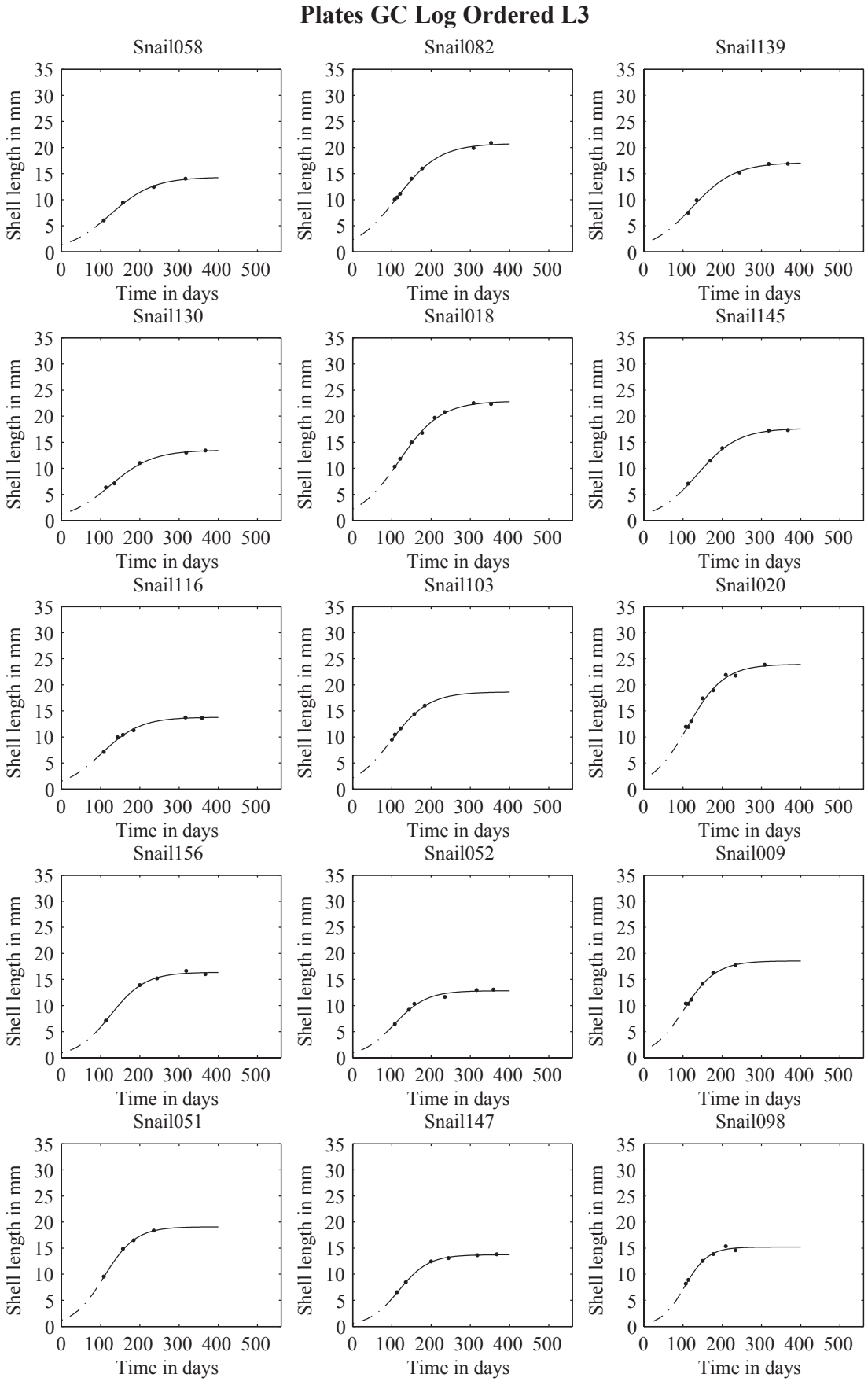

Plates GC Log Ordered L3
Snail031
Snail112
Snail064
Snail029
Snail083
Snail134
Snail067
Snail201
Snail136
Snail065
Snail095
Snail056
Snail061
Snail047
Snail111
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

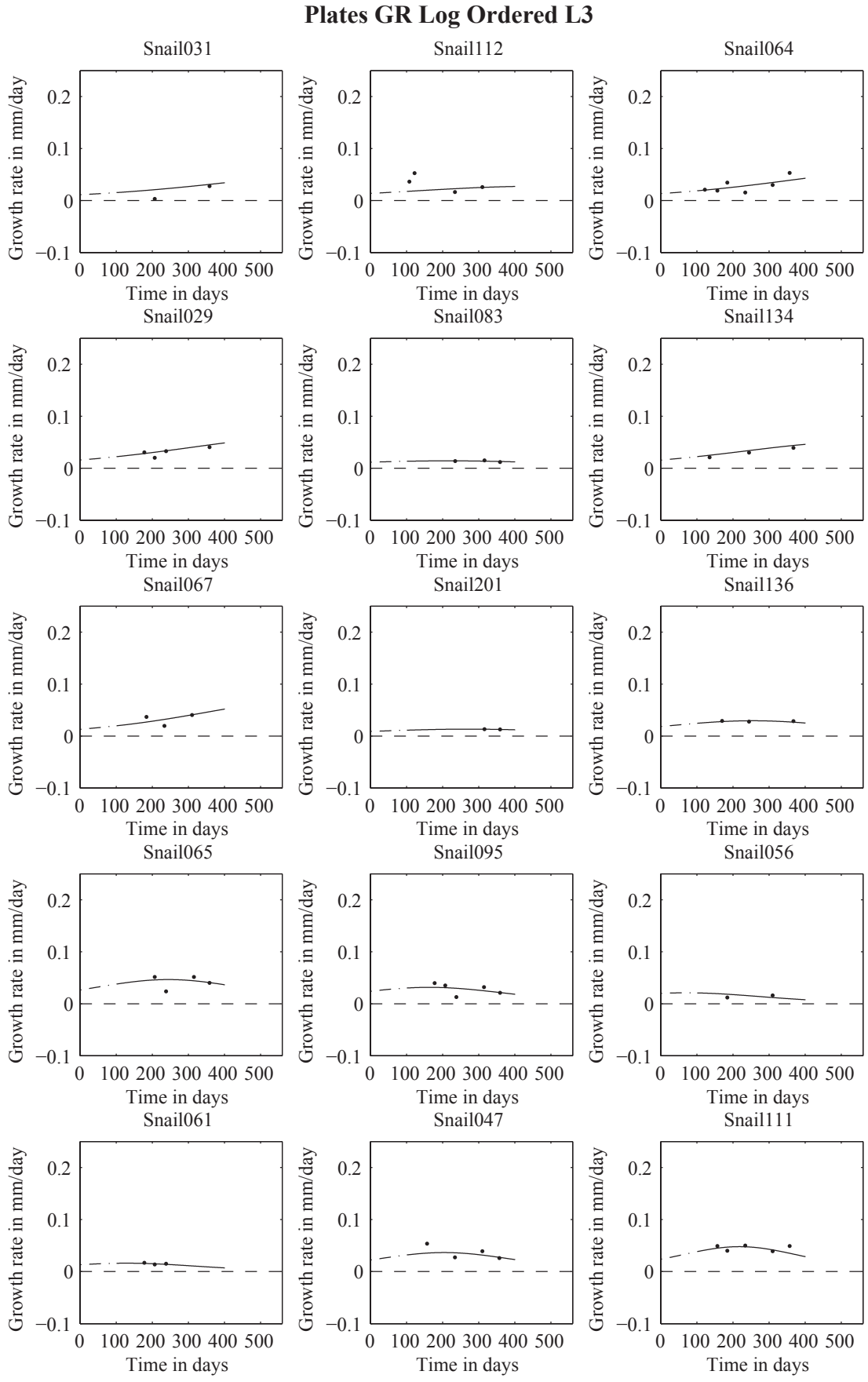

Plates GC Log Ordered L3
Snail023
Snail053
Snail010
Snail073
Snail096
Snail007
Snail006
Snail044
Snail063
Snail057
Snail144
Snail036
Snail090
Snail086
Snail045
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

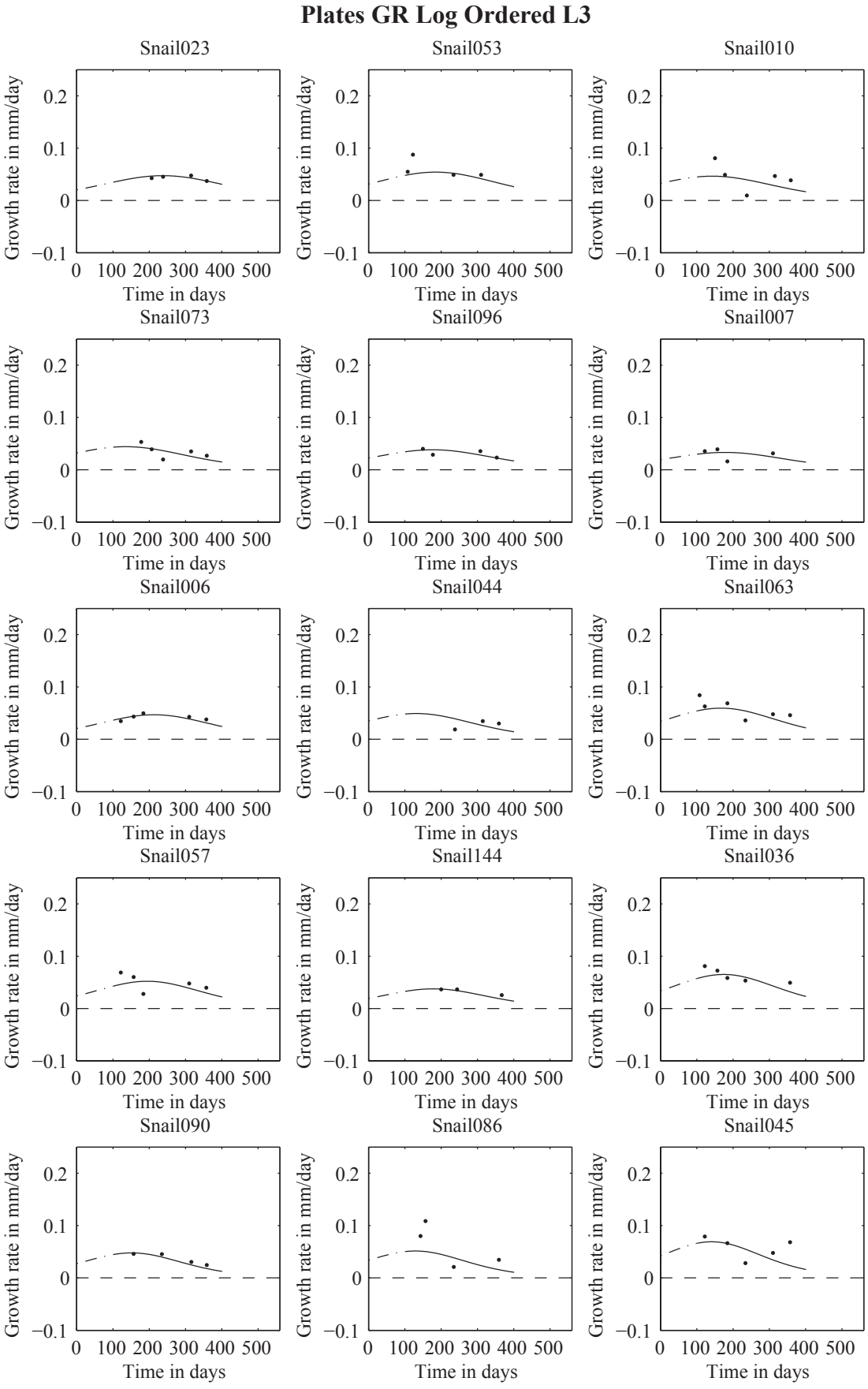

Plates GC Log Ordered L3
Snail088
Snail092
Snail028
Snail153
Snail043
Snail141
Snail002
Snail078
Snail150
Snail072
Snail152
Snail034
Snail012
Snail008
Snail131
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

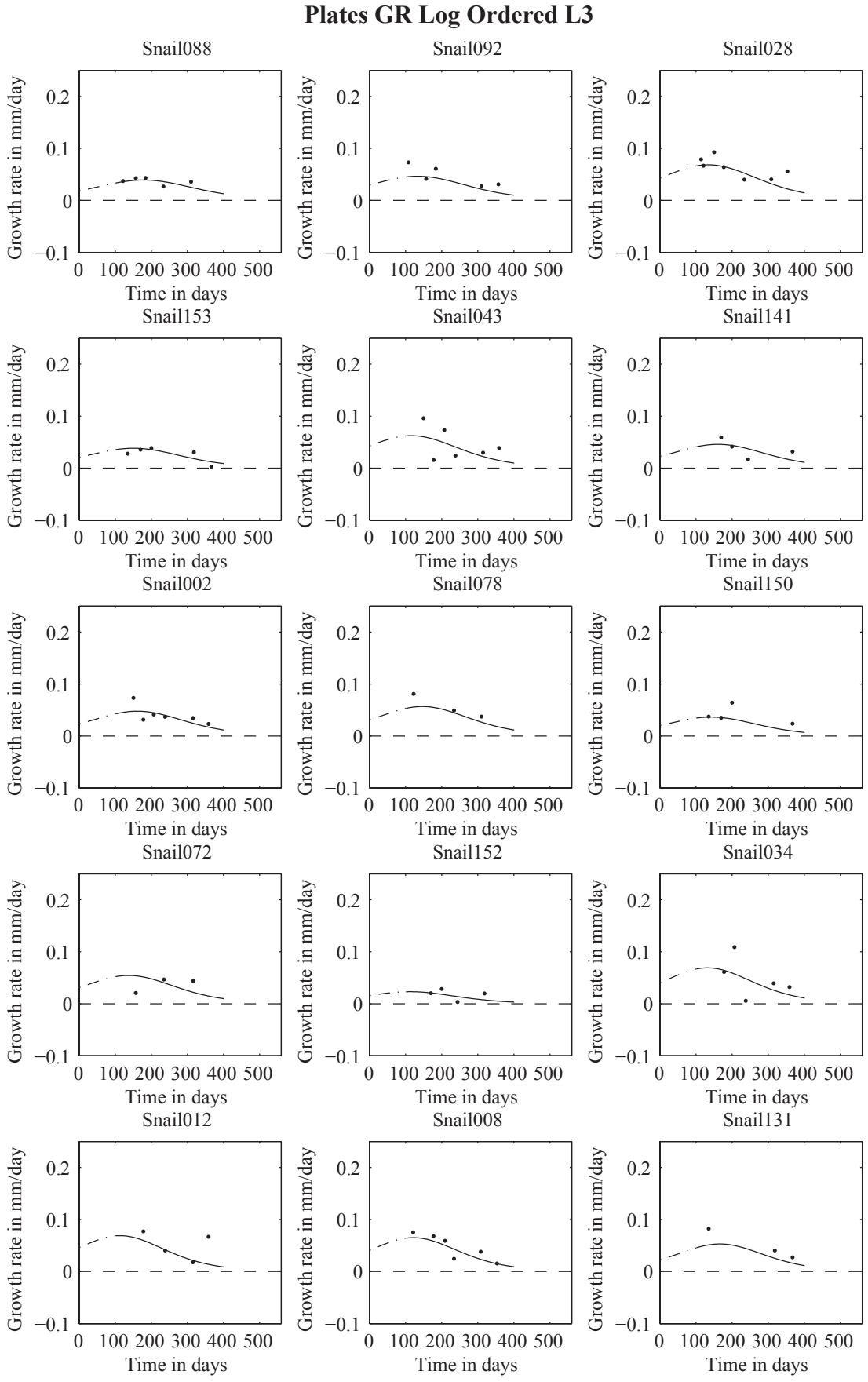

Plates GC Log Ordered L3
Snail104
Snail120
Snail133
Snail149
Snail140
Snail035
Snail062
Snail021
Snail109
Snail143
Snail066
Snail046
Snail107
Snail100
Snail013
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

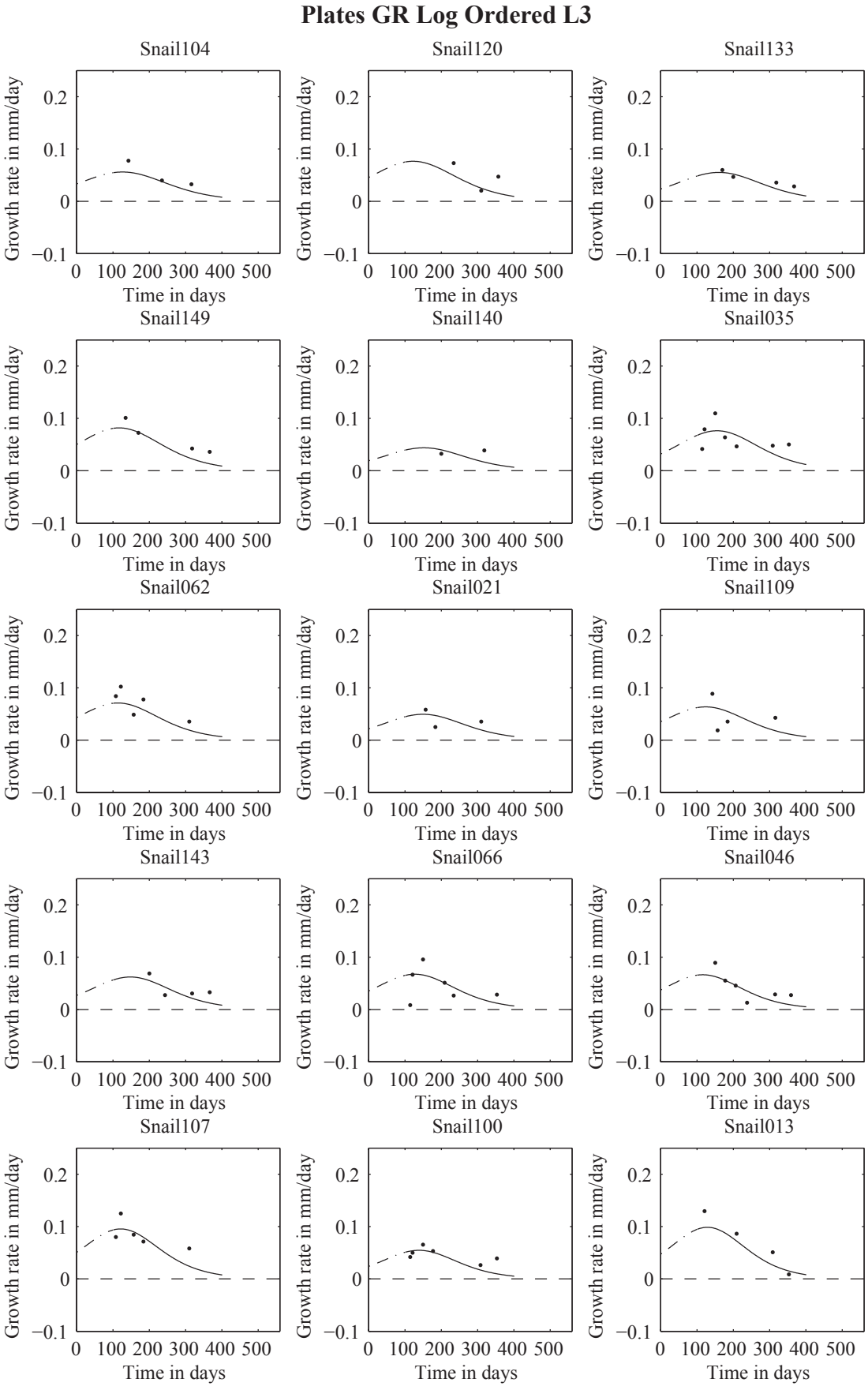

Plates GC Log Ordered L3
Snail048
Snail119
Snail032
Snail148
Snail146
Snail068
Snail038
Snail024
Snail030
Snail022
Snail026
Snail041
Snail071
Snail055
Snail142
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

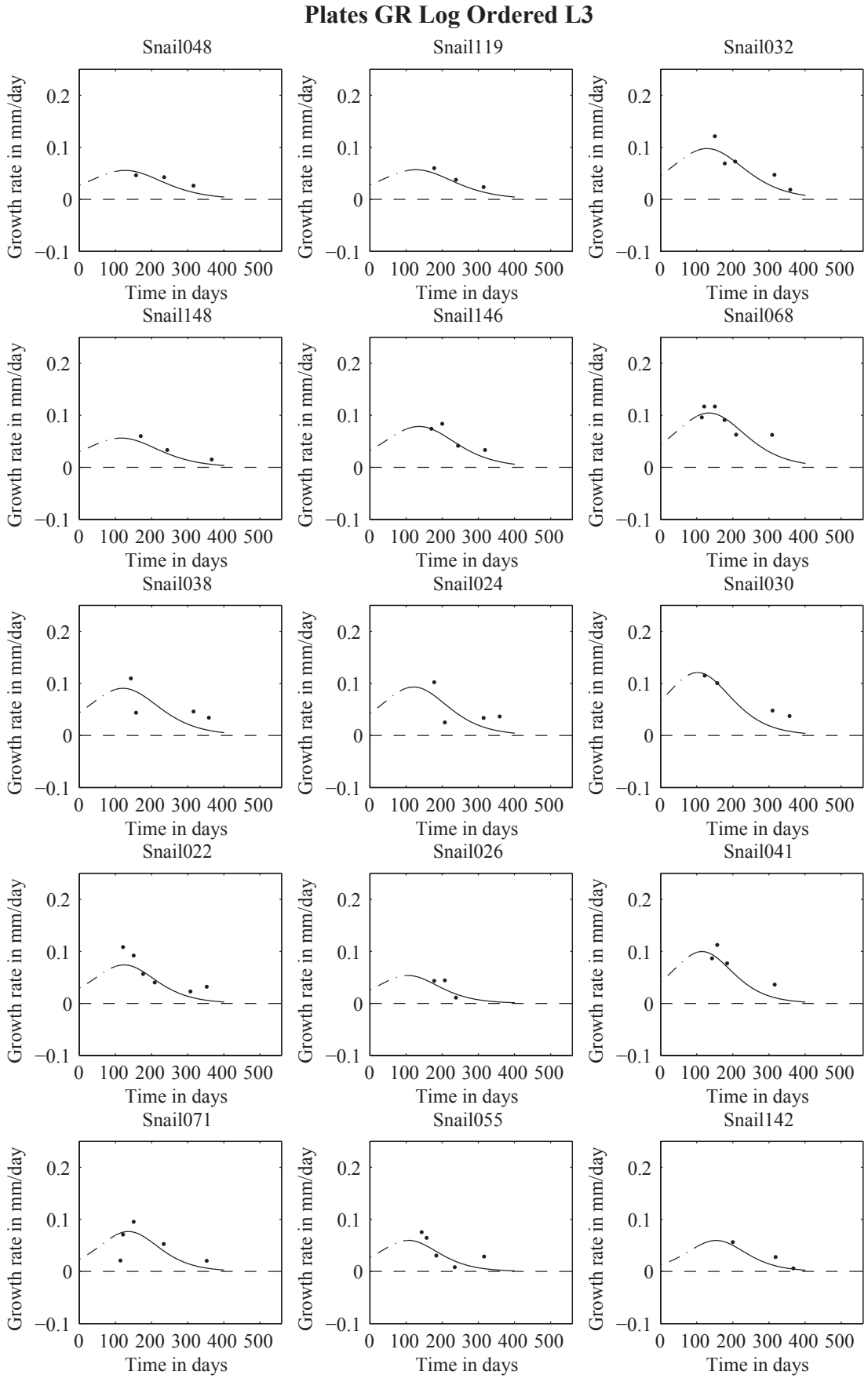

Plates GC Log Ordered L3
Snail058
Snail082
Snail139
Snail130
Snail018
Snail145
Snail116
Snail103
Snail020
Snail156
Snail052
Snail009
Snail051
Snail147
Snail098
Shell length in mm
Time in days
0 5 10 15 20 25 30 35
0 100 200 300 400 500

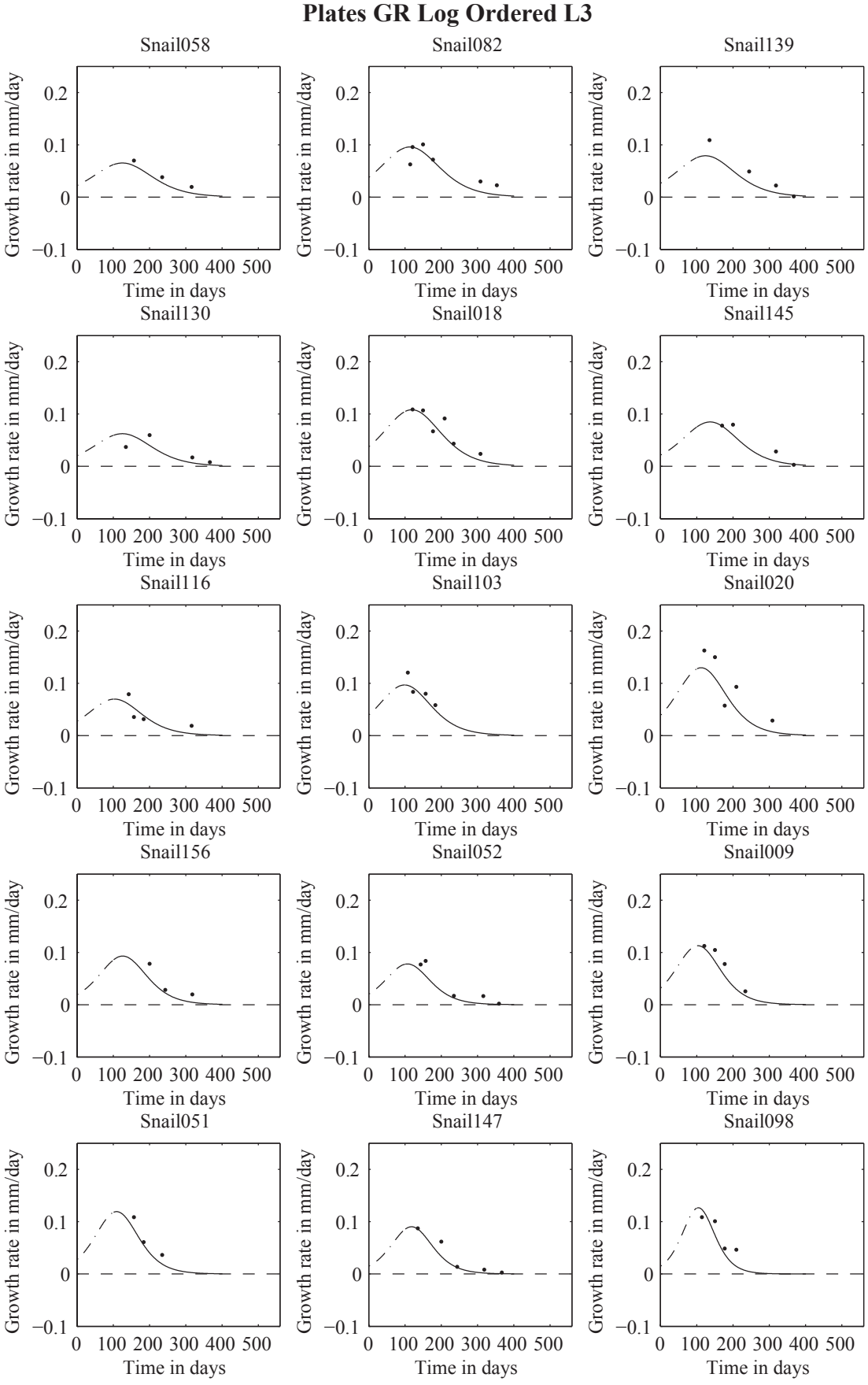

Plates GR Log Ordered L3
Snail031
Snail112
Snail064
Snail029
Snail083
Snail134
Snail067
Snail201
Snail136
Snail065
Snail095
Snail056
Snail061
Snail047
Snail111
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

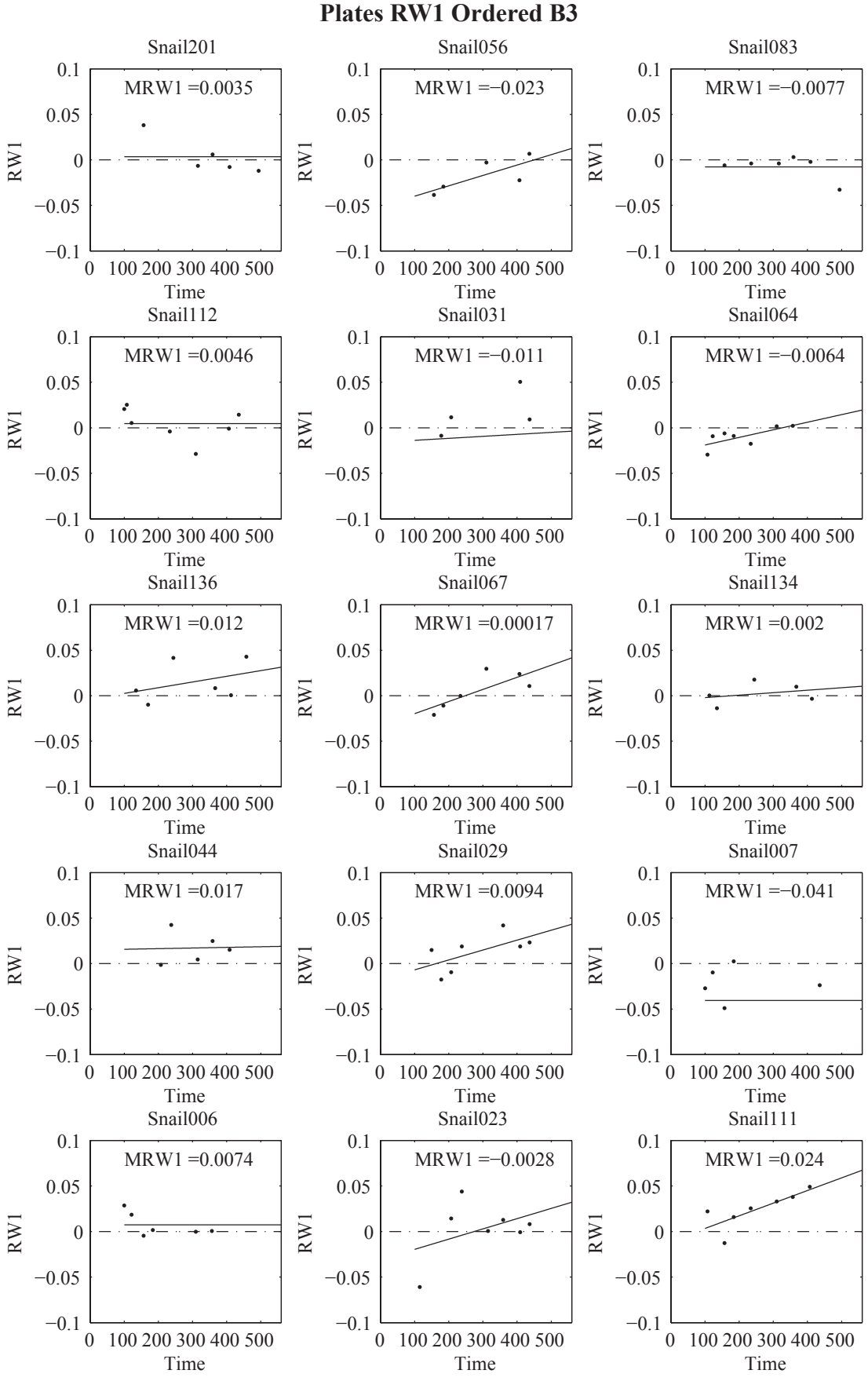

Plates GR Log Ordered L3
Snail023
Snail053
Snail010
Snail073
Snail096
Snail007
Snail006
Snail044
Snail063
Snail057
Snail144
Snail036
Snail090
Snail086
Snail045
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

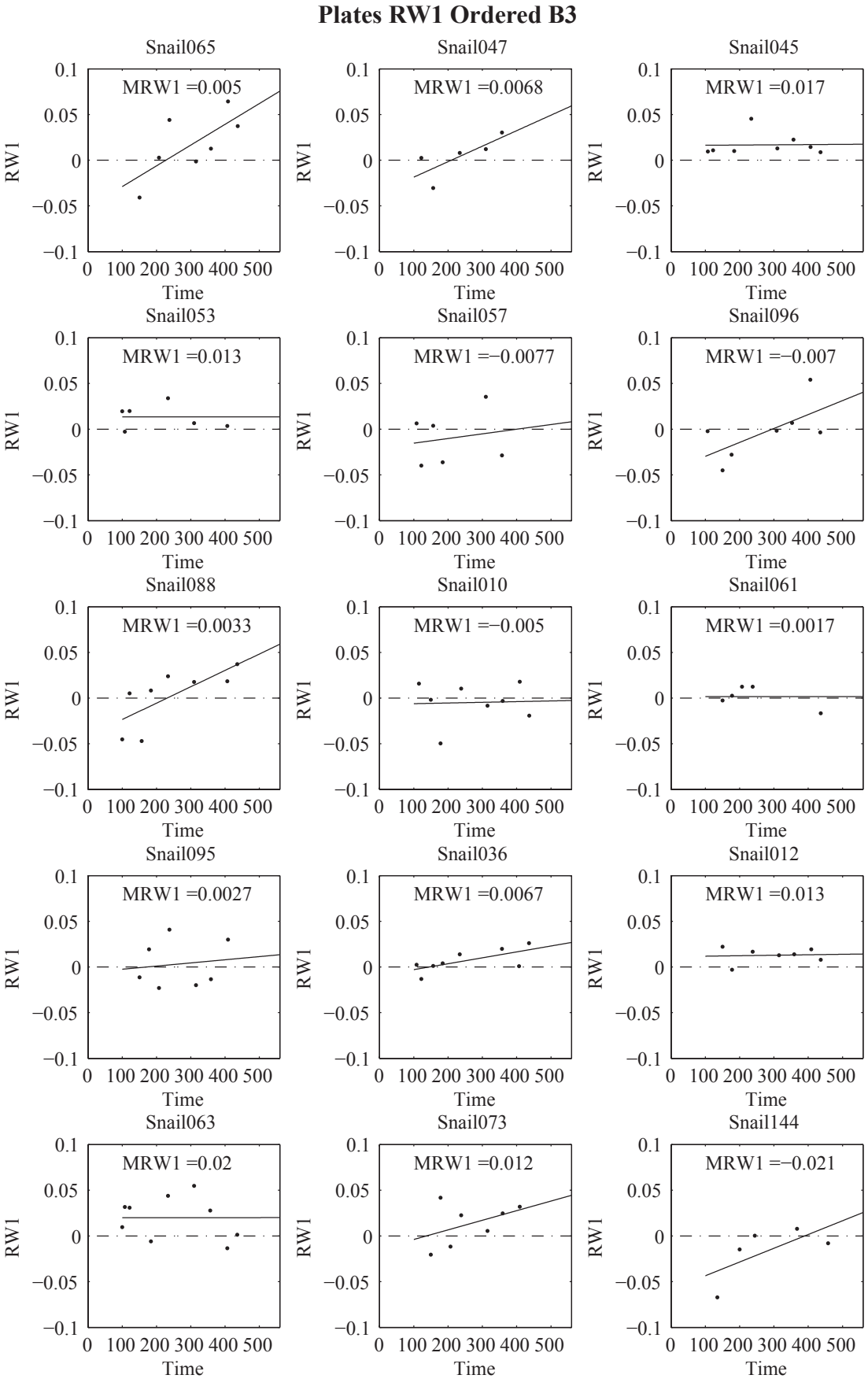

Plates GR Log Ordered L3
Snail088
Snail092
Snail028
Snail153
Snail043
Snail141
Snail002
Snail078
Snail150
Snail072
Snail152
Snail034
Snail012
Snail008
Snail131
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

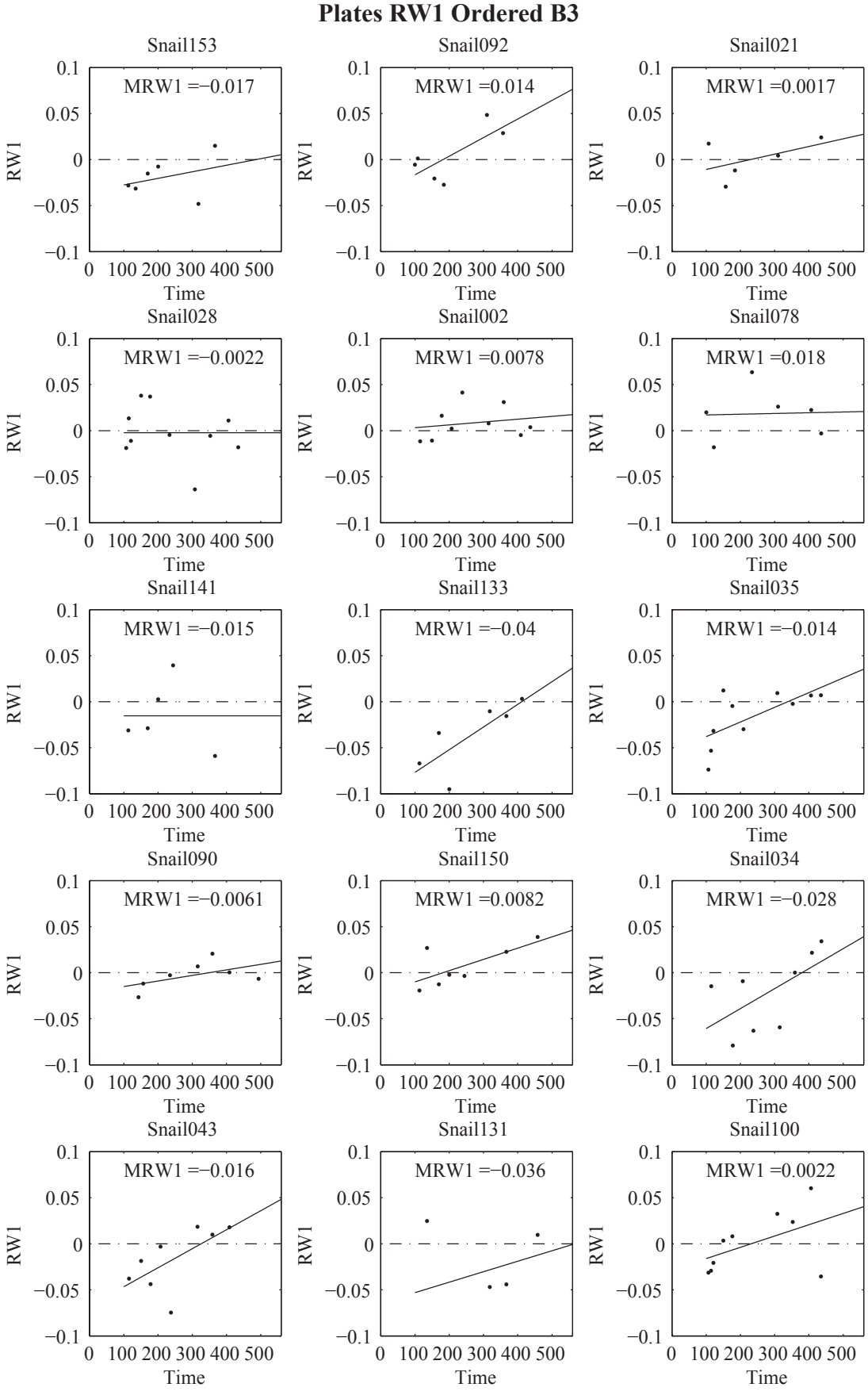

Plates GR Log Ordered L3
Snail104
Snail120
Snail133
Snail149
Snail140
Snail035
Snail062
Snail021
Snail109
Snail143
Snail066
Snail046
Snail107
Snail100
Snail013
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

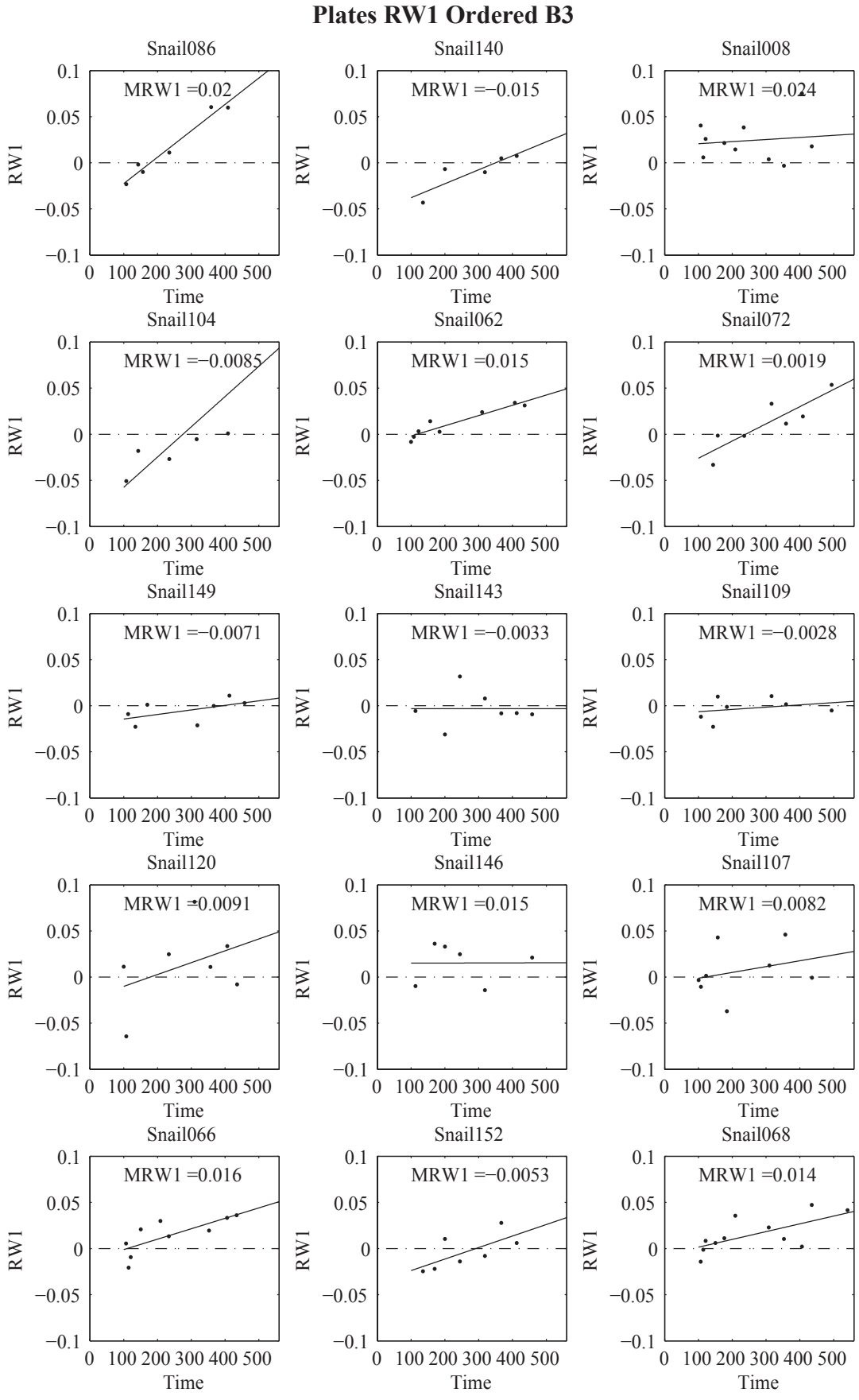

Plates GR Log Ordered L3
Snail048
Snail119
Snail032
Snail148
Snail146
Snail068
Snail038
Snail024
Snail030
Snail022
Snail026
Snail041
Snail071
Snail055
Snail142
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

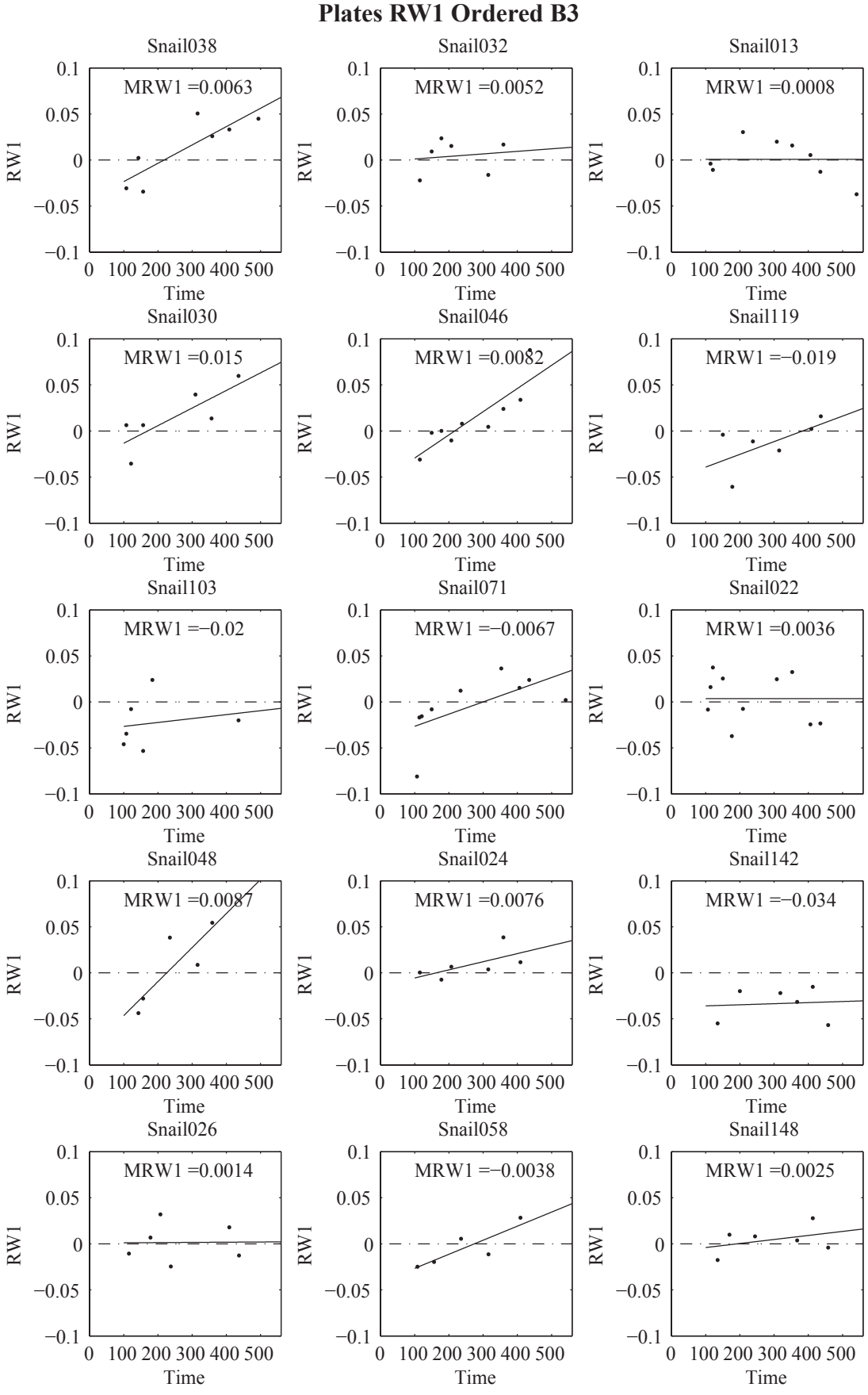

Plates GR Log Ordered L3
Snail058
Snail082
Snail139
Snail130
Snail018
Snail145
Snail116
Snail103
Snail020
Snail156
Snail052
Snail009
Snail051
Snail147
Snail098
Growth rate in mm/day
Time in days
0.2
0.1
0
−0.1
0 100 200 300 400 500

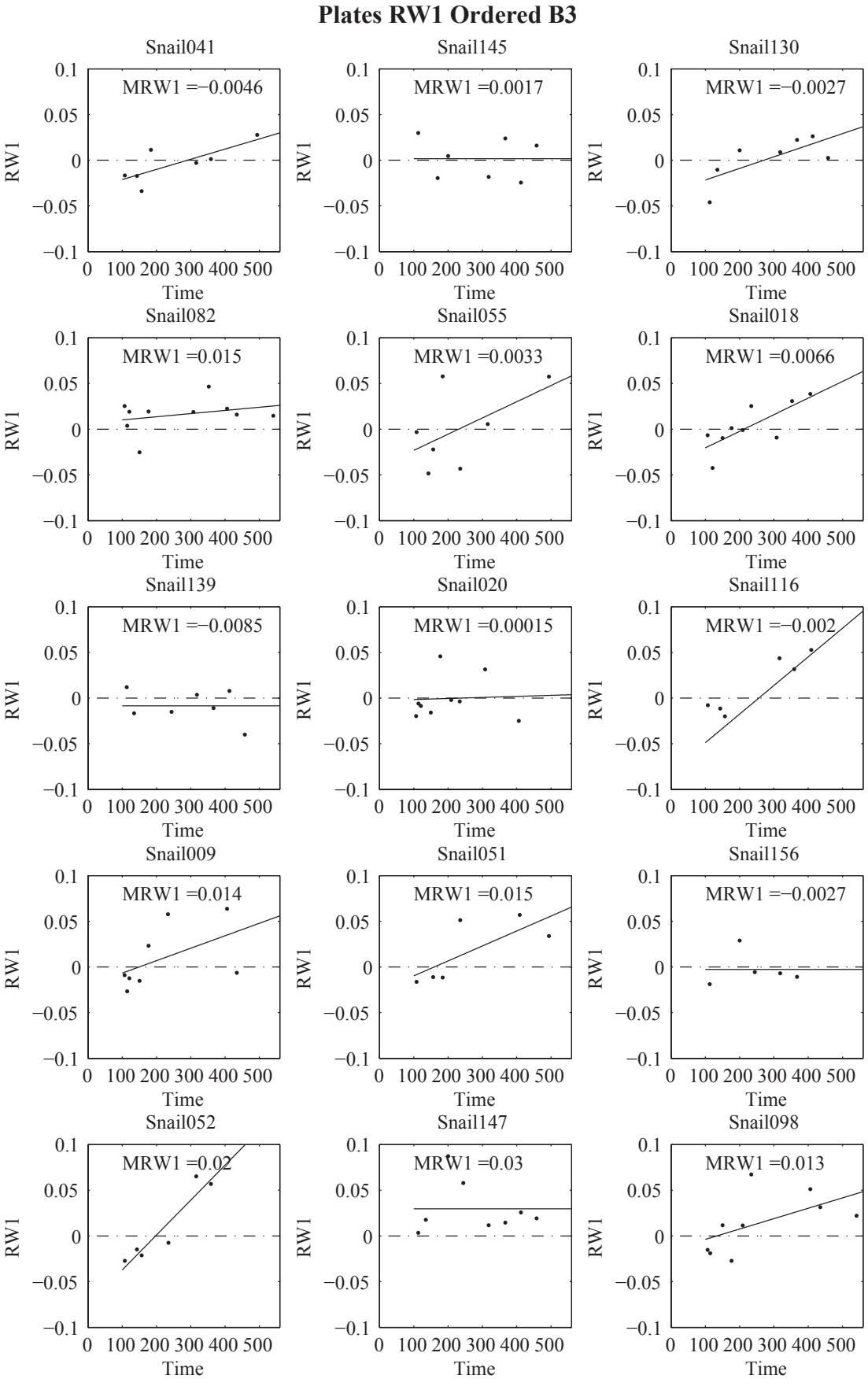

Plates RW1 Ordered B3
Snail201
MRW1 =0.0035
Snail056
MRW1 =−0.023
Snail083
MRW1 =−0.0077
Snail112
MRW1 =0.0046
Snail031
MRW1 =−0.011
Snail064
MRW1 =−0.0064
Snail136
MRW1 =0.012
Snail067
MRW1 =0.00017
Snail134
MRW1 =0.002
Snail044
MRW1 =0.017
Snail029
MRW1 =0.0094
Snail007
MRW1 =−0.041
Snail006
MRW1 =0.0074
Snail023
MRW1 =−0.0028
Snail111
MRW1 =0.024
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

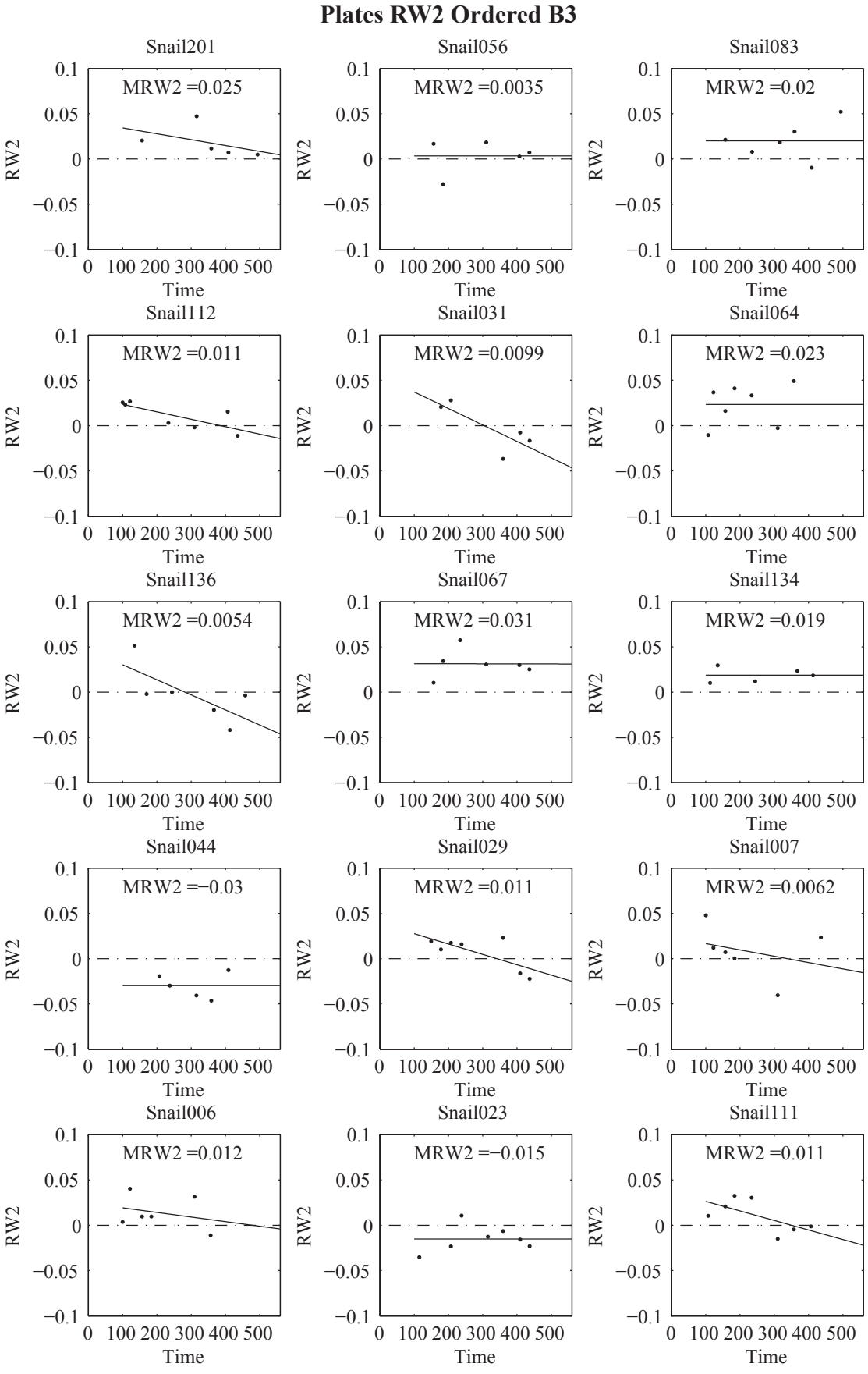

Plates RW1 Ordered B3
Snail065
MRW1 =0.005
Snail047
MRW1 =0.0068
Snail045
MRW1 =0.017
Snail053
MRW1 =0.013
Snail057
MRW1 =−0.0077
Snail096
MRW1 =−0.007
Snail088
MRW1 =0.0033
Snail010
MRW1 =−0.005
Snail061
MRW1 =0.0017
Snail095
MRW1 =0.0027
Snail036
MRW1 =0.0067
Snail012
MRW1 =0.013
Snail063
MRW1 =0.02
Snail073
MRW1 =0.012
Snail144
MRW1 =−0.021
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

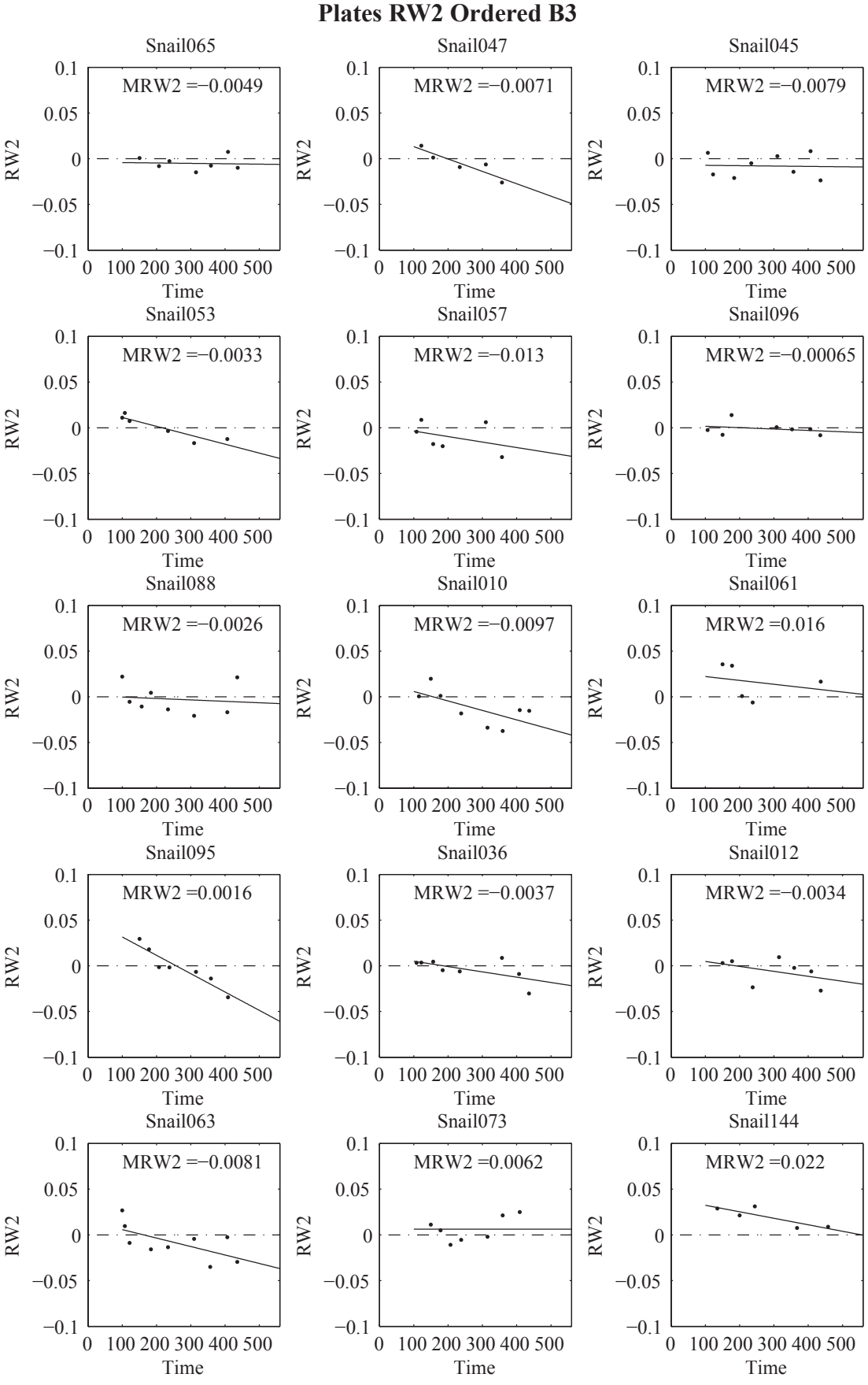

Plates RW1 Ordered B3
Snail153
MRW1 =−0.017
Snail092
MRW1 =0.014
Snail021
MRW1 =0.0017
Snail028
MRW1 =−0.0022
Snail002
MRW1 =0.0078
Snail078
MRW1 =0.018
Snail141
MRW1 =−0.015
Snail133
MRW1 =−0.04
Snail035
MRW1 =−0.014
Snail090
MRW1 =−0.0061
Snail150
MRW1 =0.0082
Snail034
MRW1 =−0.028
Snail043
MRW1 =−0.016
Snail131
MRW1 =−0.036
Snail100
MRW1 =0.0022
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

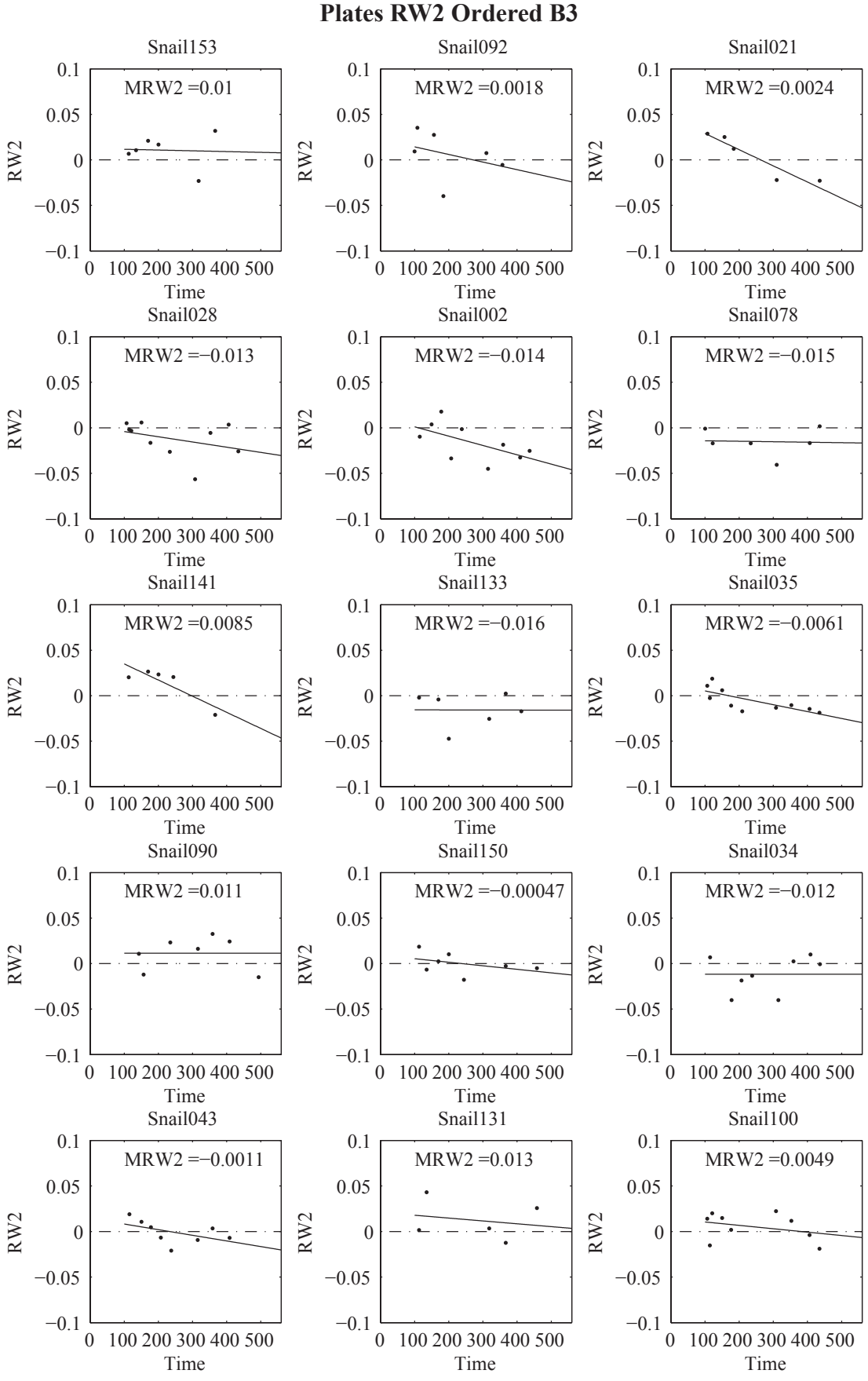

Plates RW1 Ordered B3
Snail086
MRW1 =0.02
Snail140
MRW1 =−0.015
Snail008
MRW1 =0.024
Snail104
MRW1 =−0.0085
Snail062
MRW1 =0.015
Snail072
MRW1 =0.0019
Snail149
MRW1 =−0.0071
Snail143
MRW1 =−0.0033
Snail109
MRW1 =−0.0028
Snail120
MRW1 =0.0091
Snail146
MRW1 =0.015
Snail107
MRW1 =0.0082
Snail066
MRW1 =0.016
Snail152
MRW1 =−0.0053
Snail068
MRW1 =0.014
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

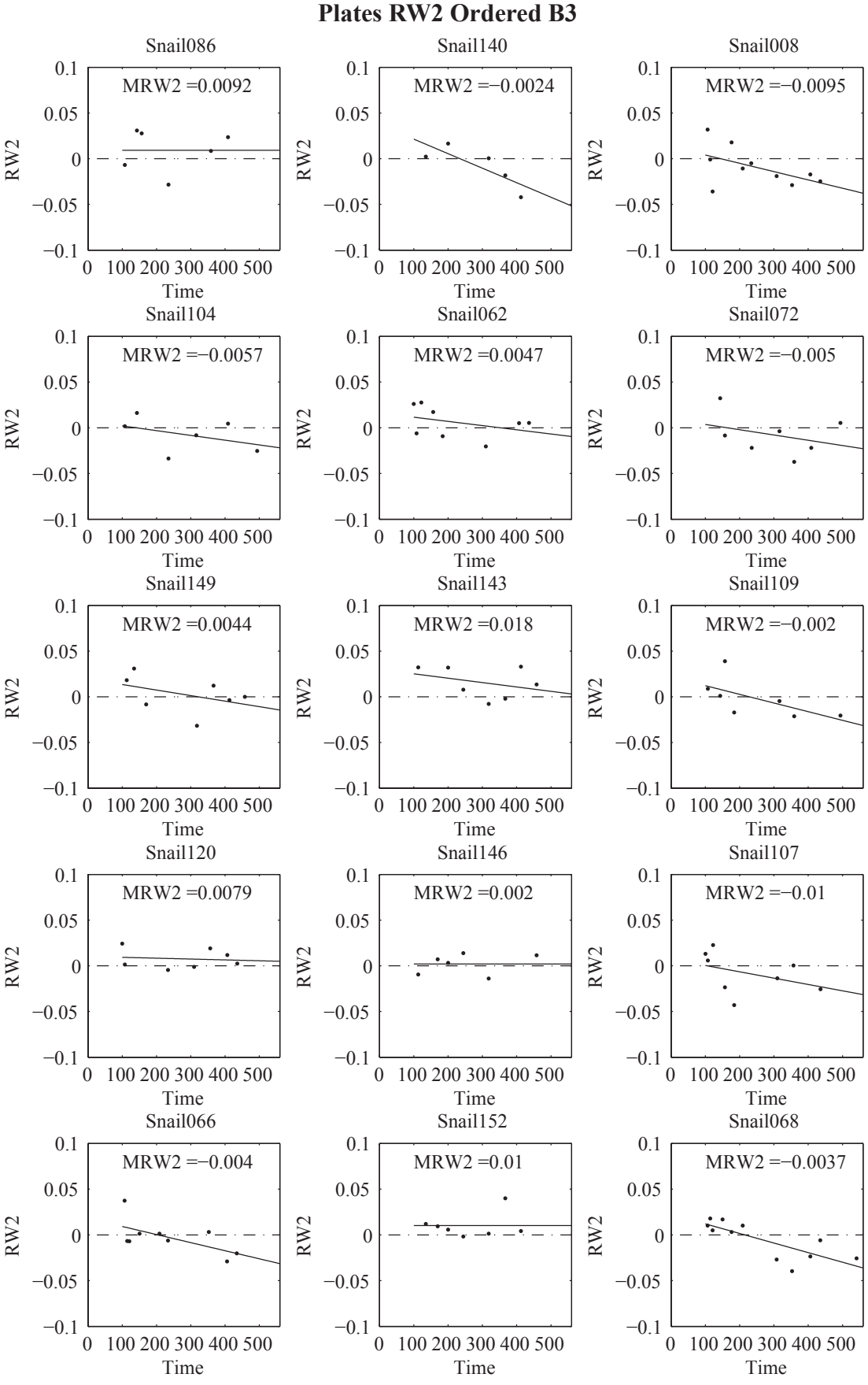

Plates RW1 Ordered B3
Snail038
MRW1 =0.0063
Snail032
MRW1 =0.0052
Snail013
MRW1 =0.0008
Snail030
MRW1 =0.015
Snail046
MRW1 =0.0082
Snail119
MRW1 =−0.019
Snail103
MRW1 =−0.02
Snail071
MRW1 =−0.0067
Snail022
MRW1 =0.0036
Snail048
MRW1 =0.0087
Snail024
MRW1 =0.0076
Snail142
MRW1 =−0.034
Snail026
MRW1 =0.0014
Snail058
MRW1 =−0.0038
Snail148
MRW1 =0.0025
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

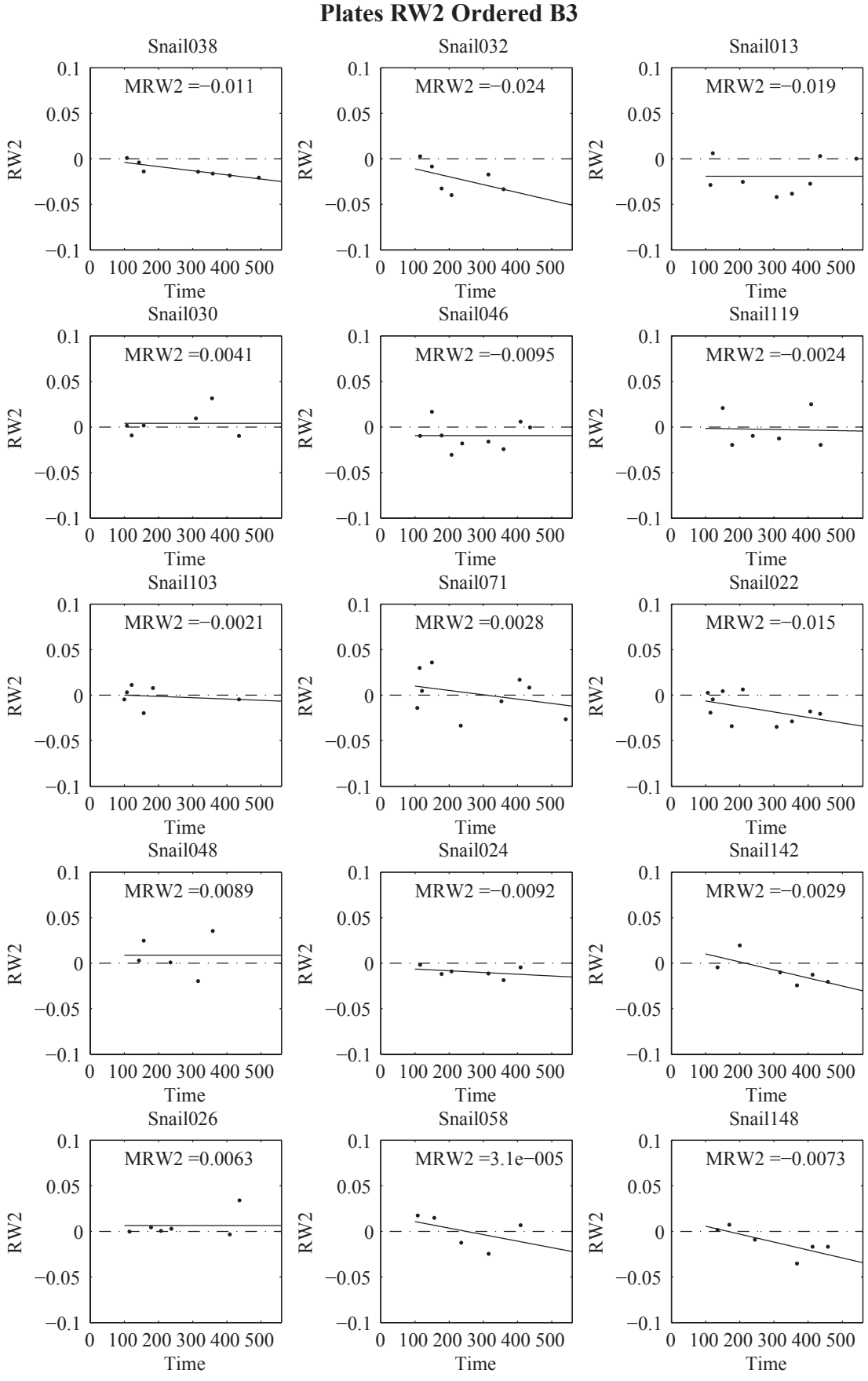

Plates RW1 Ordered B3
Snail041
MRW1 =−0.0046
Snail145
MRW1 =0.0017
Snail130
MRW1 =−0.0027
Snail082
MRW1 =0.015
Snail055
MRW1 =0.0033
Snail018
MRW1 =0.0066
Snail139
MRW1 =−0.0085
Snail020
MRW1 =0.00015
Snail116
MRW1 =−0.002
Snail009
MRW1 =0.014
Snail051
MRW1 =0.015
Snail156
MRW1 =−0.0027
Snail052
MRW1 =0.02
Snail147
MRW1 =0.03
Snail098
MRW1 =0.013
RW1
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

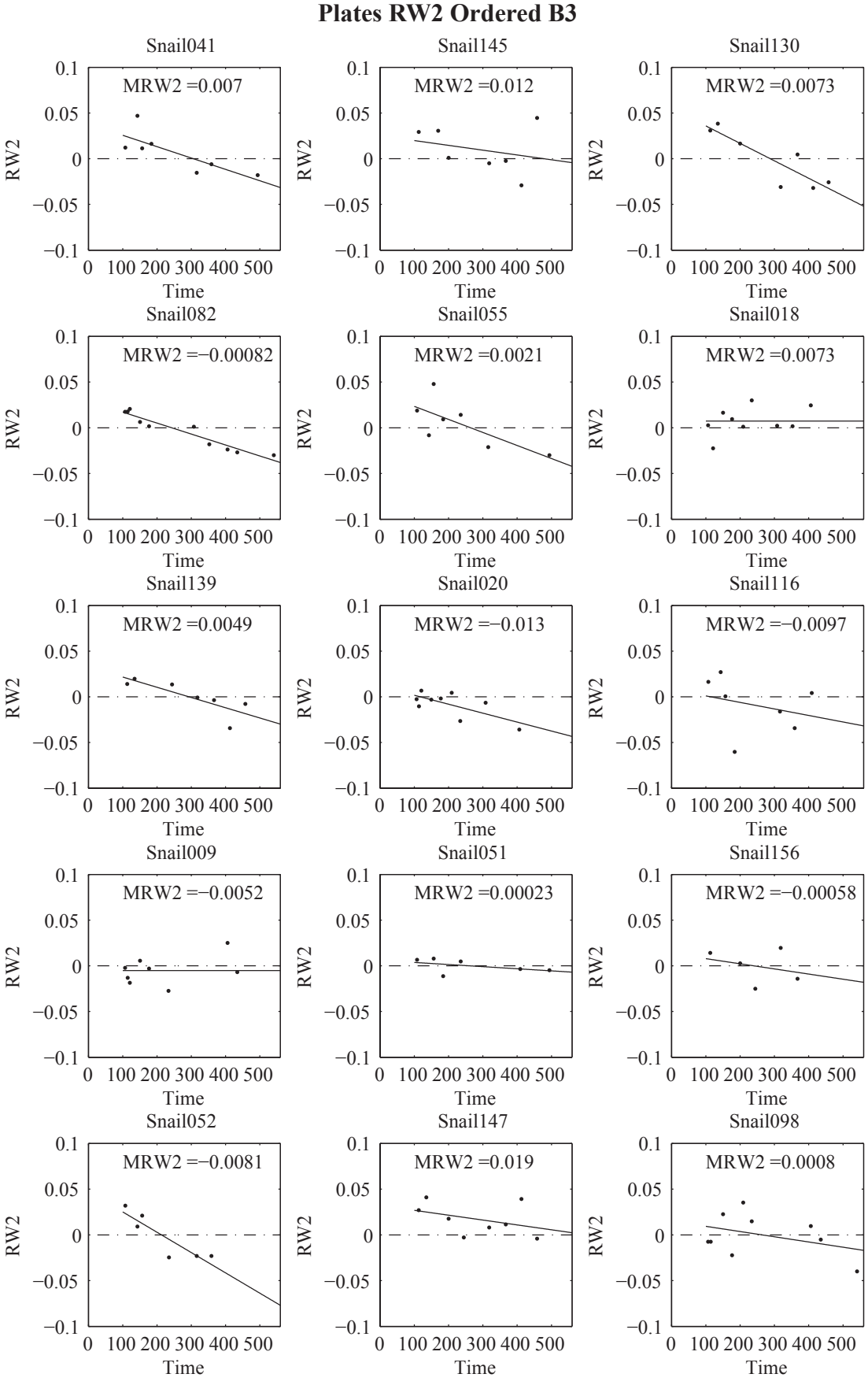

Plates RW2 Ordered B3
Snail201
MRW2 =0.025
Snail056
MRW2 =0.0035
Snail083
MRW2 =0.02
Snail112
MRW2 =0.011
Snail031
MRW2 =0.0099
Snail064
MRW2 =0.023
Snail136
MRW2 =0.0054
Snail067
MRW2 =0.031
Snail134
MRW2 =0.019
Snail044
MRW2 =−0.03
Snail029
MRW2 =0.011
Snail007
MRW2 =0.0062
Snail006
MRW2 =0.012
Snail023
MRW2 =−0.015
Snail111
MRW2 =0.011
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

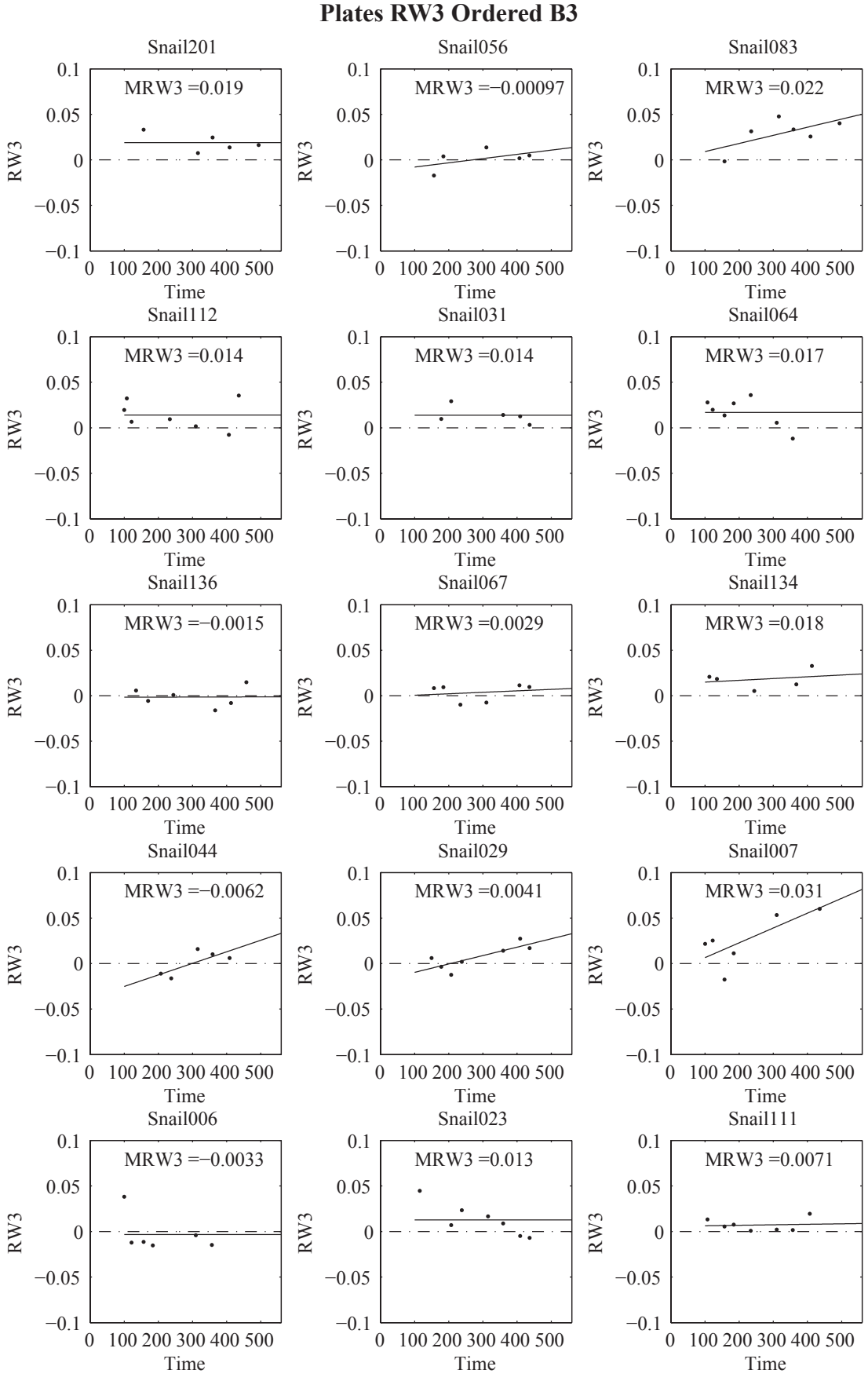

Plates RW2 Ordered B3
Snail065
MRW2 =−0.0049
Snail047
MRW2 =−0.0071
Snail045
MRW2 =−0.0079
Snail053
MRW2 =−0.0033
Snail057
MRW2 =−0.013
Snail096
MRW2 =−0.00065
Snail088
MRW2 =−0.0026
Snail010
MRW2 =−0.0097
Snail061
MRW2 =0.016
Snail095
MRW2 =0.0016
Snail036
MRW2 =−0.0037
Snail012
MRW2 =−0.0034
Snail063
MRW2 =−0.0081
Snail073
MRW2 =0.0062
Snail144
MRW2 =0.022
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

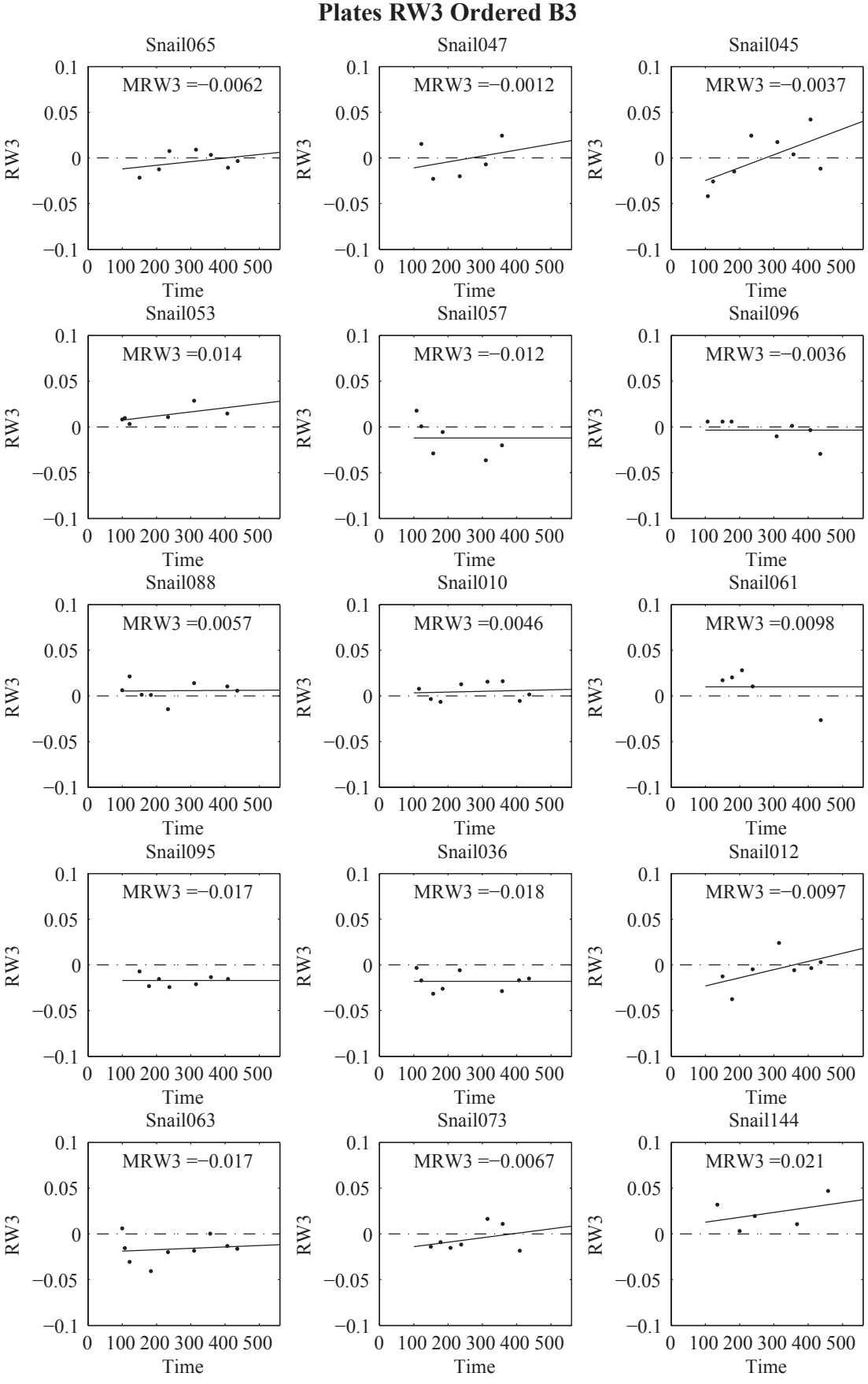

Plates RW2 Ordered B3
Snail153
MRW2 =0.01
Snail092
MRW2 =0.0018
Snail021
MRW2 =0.0024
Snail028
MRW2 =−0.013
Snail002
MRW2 =−0.014
Snail078
MRW2 =−0.015
Snail141
MRW2 =0.0085
Snail133
MRW2 =−0.016
Snail035
MRW2 =−0.0061
Snail090
MRW2 =0.011
Snail150
MRW2 =−0.00047
Snail034
MRW2 =−0.012
Snail043
MRW2 =−0.0011
Snail131
MRW2 =0.013
Snail100
MRW2 =0.0049
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

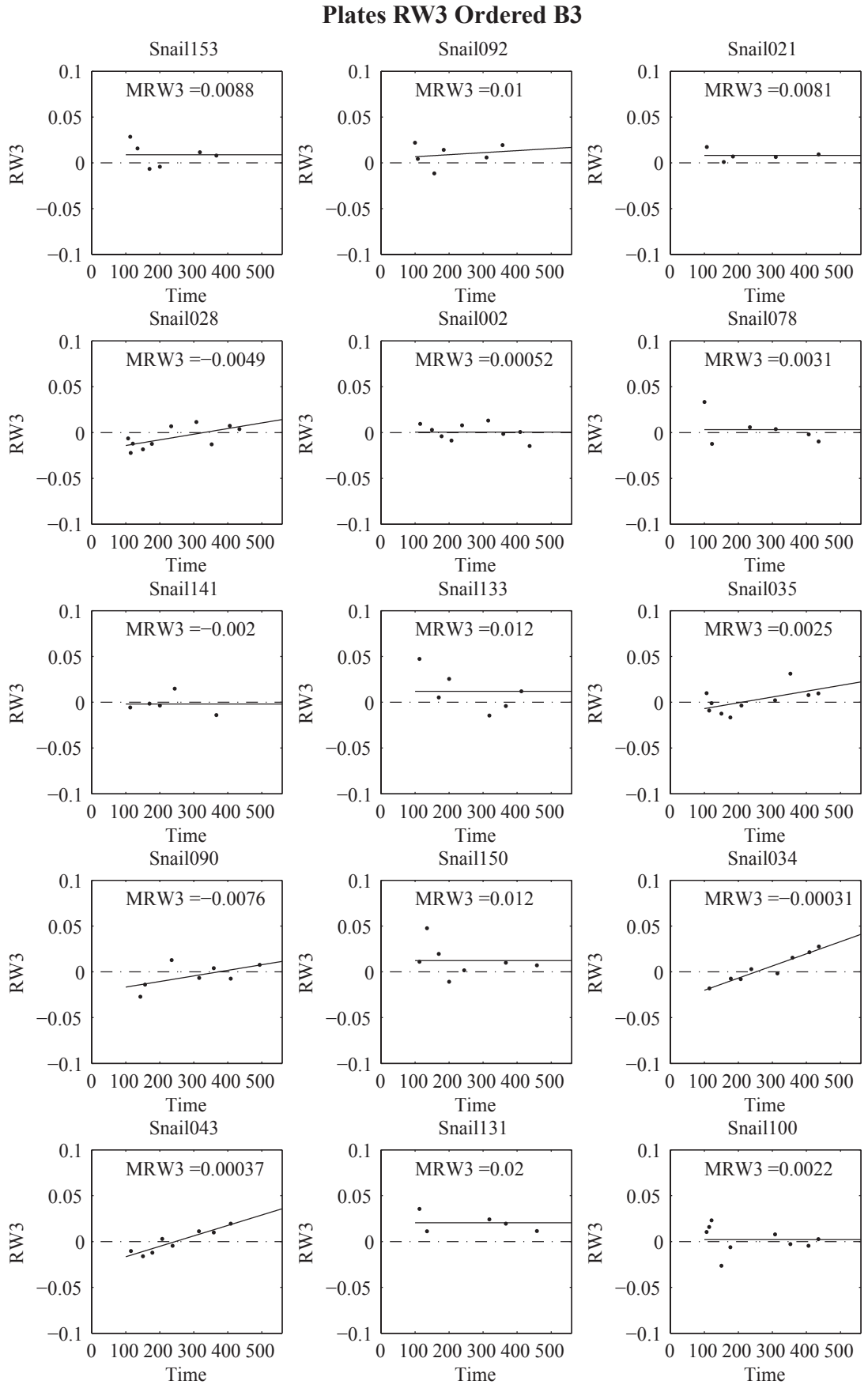

Plates RW2 Ordered B3
Snail086
MRW2 =0.0092
Snail140
MRW2 =−0.0024
Snail008
MRW2 =−0.0095
Snail104
MRW2 =−0.0057
Snail062
MRW2 =0.0047
Snail072
MRW2 =−0.005
Snail149
MRW2 =0.0044
Snail143
MRW2 =0.018
Snail109
MRW2 =−0.002
Snail120
MRW2 =0.0079
Snail146
MRW2 =0.002
Snail107
MRW2 =−0.01
Snail066
MRW2 =−0.004
Snail152
MRW2 =0.01
Snail068
MRW2 =−0.0037
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

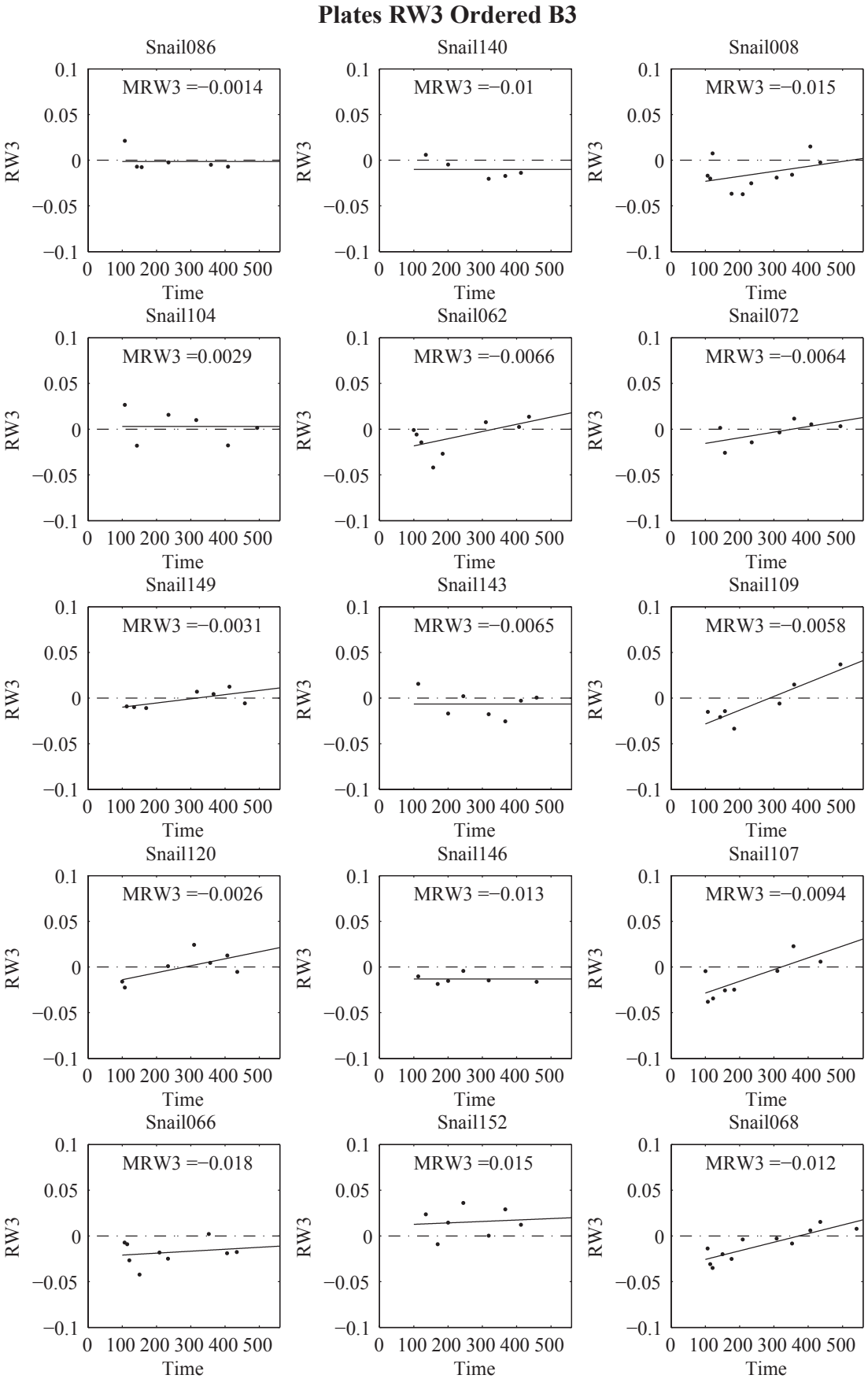

Plates RW2 Ordered B3
Snail038
MRW2 =−0.011
Snail032
MRW2 =−0.024
Snail013
MRW2 =−0.019
Snail030
MRW2 =0.0041
Snail046
MRW2 =−0.0095
Snail119
MRW2 =−0.0024
Snail103
MRW2 =−0.0021
Snail071
MRW2 =0.0028
Snail022
MRW2 =−0.015
Snail048
MRW2 =0.0089
Snail024
MRW2 =−0.0092
Snail142
MRW2 =−0.0029
Snail026
MRW2 =0.0063
Snail058
MRW2 =3.1e−005
Snail148
MRW2 =−0.0073
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

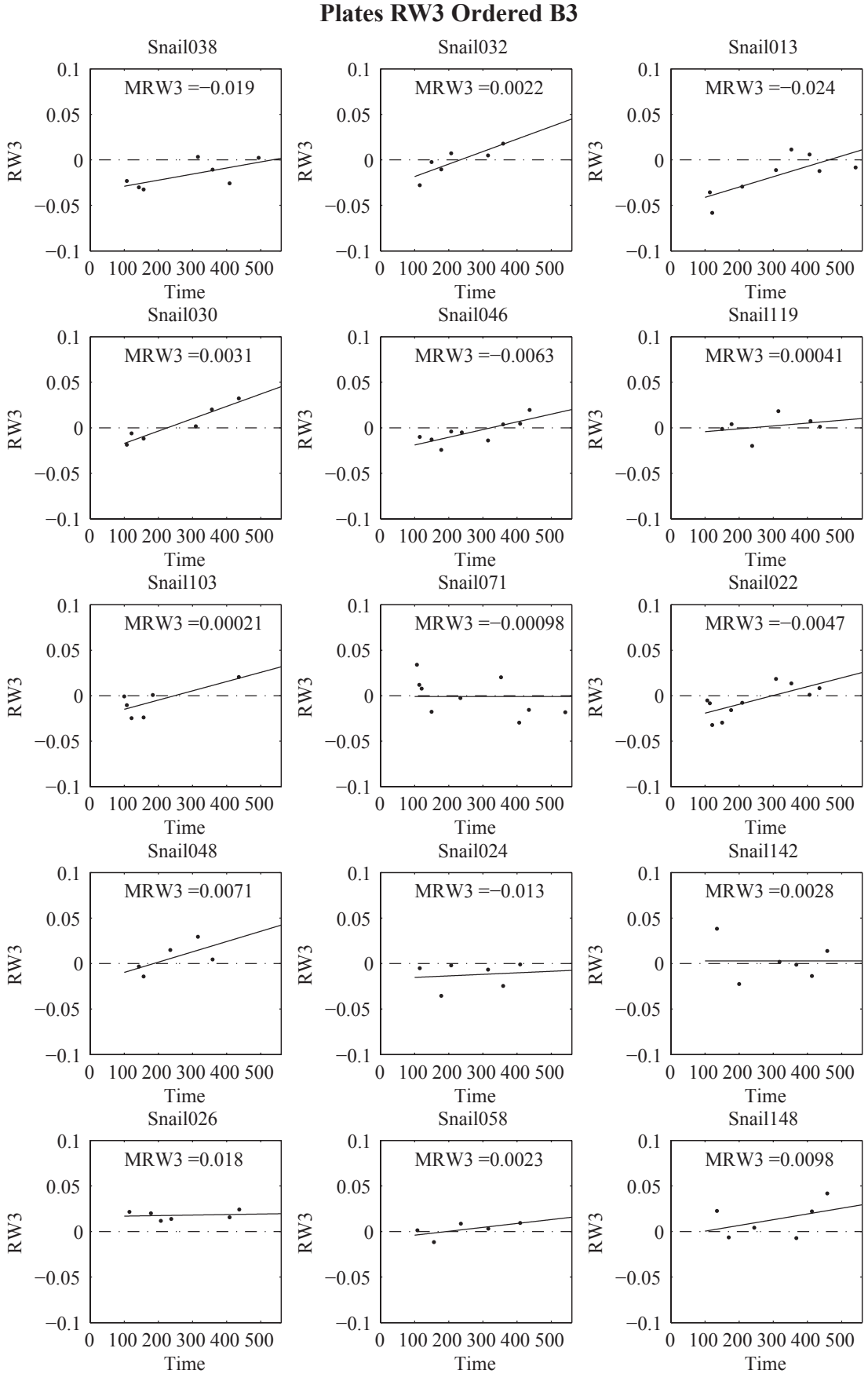

Plates RW2 Ordered B3
Snail041
MRW2 =0.007
Snail145
MRW2 =0.012
Snail130
MRW2 =0.0073
Snail082
MRW2 =−0.00082
Snail055
MRW2 =0.0021
Snail018
MRW2 =0.0073
Snail139
MRW2 =0.0049
Snail020
MRW2 =−0.013
Snail116
MRW2 =−0.0097
Snail009
MRW2 =−0.0052
Snail051
MRW2 =0.00023
Snail156
MRW2 =−0.00058
Snail052
MRW2 =−0.0081
Snail147
MRW2 =0.019
Snail098
MRW2 =0.0008
RW2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

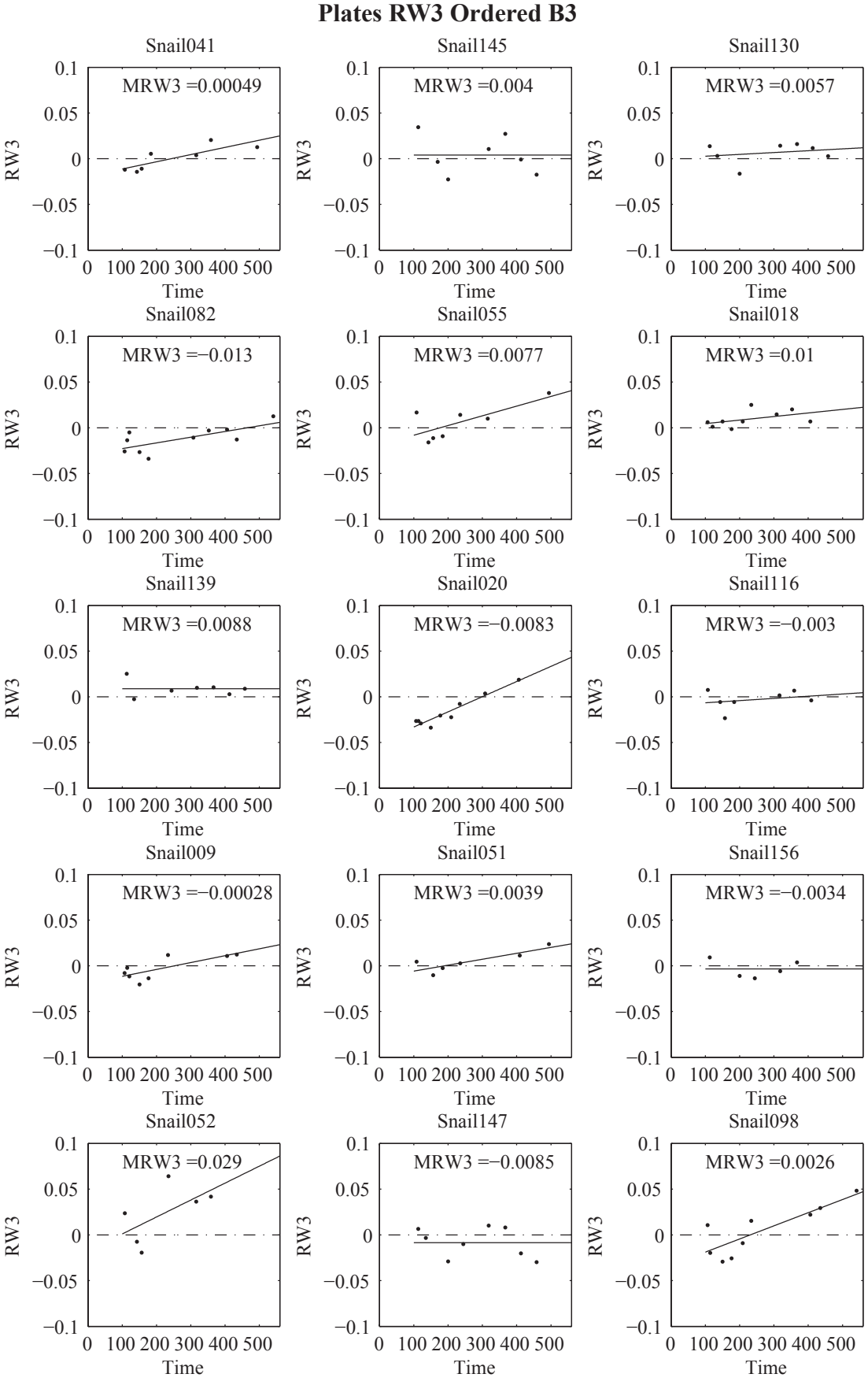

Plates RW3 Ordered B3
Snail201
MRW3 =0.019
Snail056
MRW3 =−0.00097
Snail083
MRW3 =0.022
Snail112
MRW3 =0.014
Snail031
MRW3 =0.014
Snail064
MRW3 =0.017
Snail136
MRW3 =−0.0015
Snail067
MRW3 =0.0029
Snail134
MRW3 =0.018
Snail044
MRW3 =−0.0062
Snail029
MRW3 =0.0041
Snail007
MRW3 =0.031
Snail006
MRW3 =−0.0033
Snail023
MRW3 =0.013
Snail111
MRW3 =0.0071
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

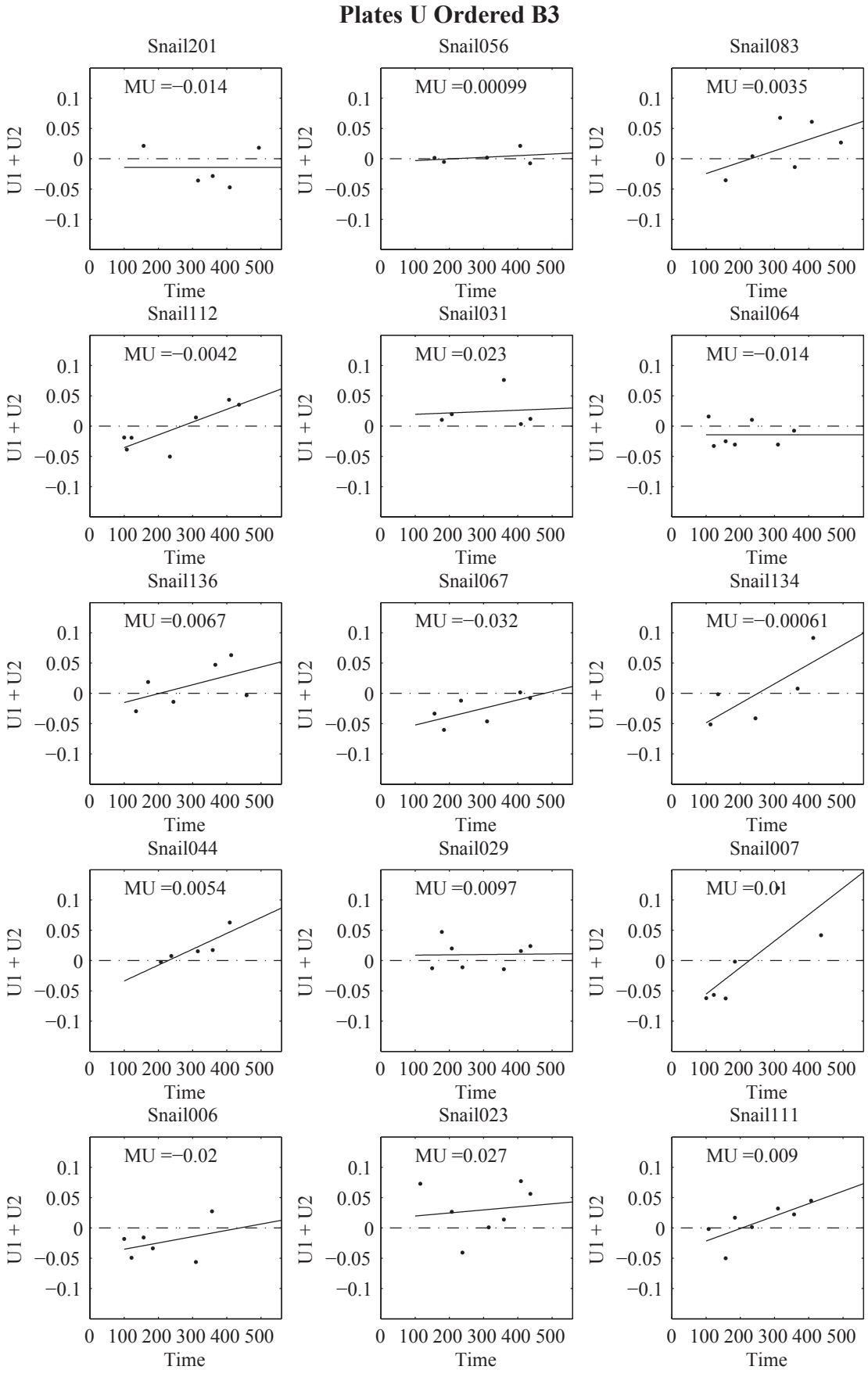

Plates RW3 Ordered B3
Snail065
MRW3 =−0.0062
Snail047
MRW3 =−0.0012
Snail045
MRW3 =−0.0037
Snail053
MRW3 =0.014
Snail057
MRW3 =−0.012
Snail096
MRW3 =−0.0036
Snail088
MRW3 =0.0057
Snail010
MRW3 =0.0046
Snail061
MRW3 =0.0098
Snail095
MRW3 =−0.017
Snail036
MRW3 =−0.018
Snail012
MRW3 =−0.0097
Snail063
MRW3 =−0.017
Snail073
MRW3 =−0.0067
Snail144
MRW3 =0.021
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

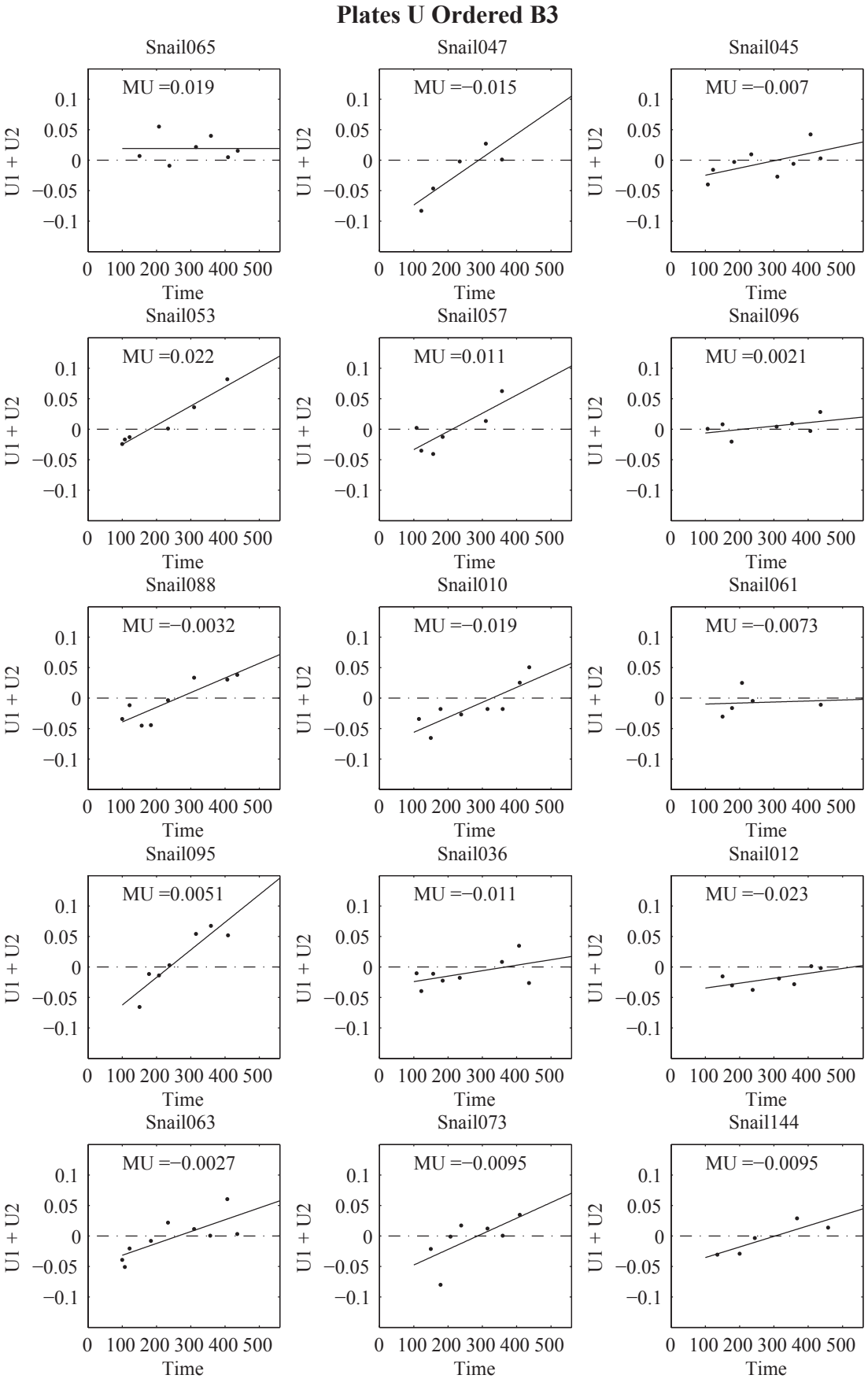

Plates RW3 Ordered B3
Snail153
MRW3 =0.0088
Snail092
MRW3 =0.01
Snail021
MRW3 =0.0081
Snail028
MRW3 =−0.0049
Snail002
MRW3 =0.00052
Snail078
MRW3 =0.0031
Snail141
MRW3 =−0.002
Snail133
MRW3 =0.012
Snail035
MRW3 =0.0025
Snail090
MRW3 =−0.0076
Snail150
MRW3 =0.012
Snail034
MRW3 =−0.00031
Snail043
MRW3 =0.00037
Snail131
MRW3 =0.02
Snail100
MRW3 =0.0022
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

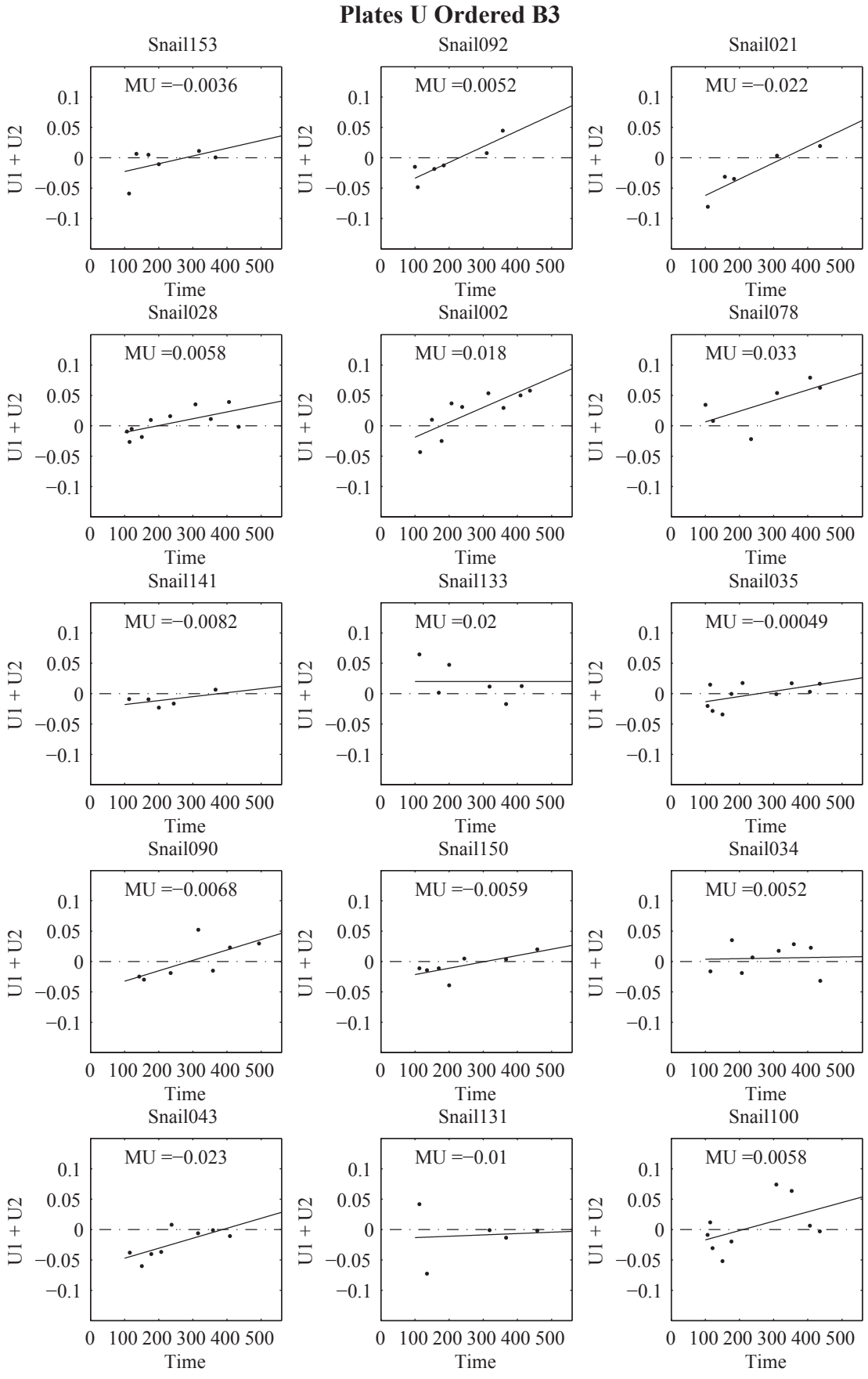

Plates RW3 Ordered B3
Snail086
MRW3 =−0.0014
Snail140
MRW3 =−0.01
Snail008
MRW3 =−0.015
Snail104
MRW3 =0.0029
Snail062
MRW3 =−0.0066
Snail072
MRW3 =−0.0064
Snail149
MRW3 =−0.0031
Snail143
MRW3 =−0.0065
Snail109
MRW3 =−0.0058
Snail120
MRW3 =−0.0026
Snail146
MRW3 =−0.013
Snail107
MRW3 =−0.0094
Snail066
MRW3 =−0.018
Snail152
MRW3 =0.015
Snail068
MRW3 =−0.012
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

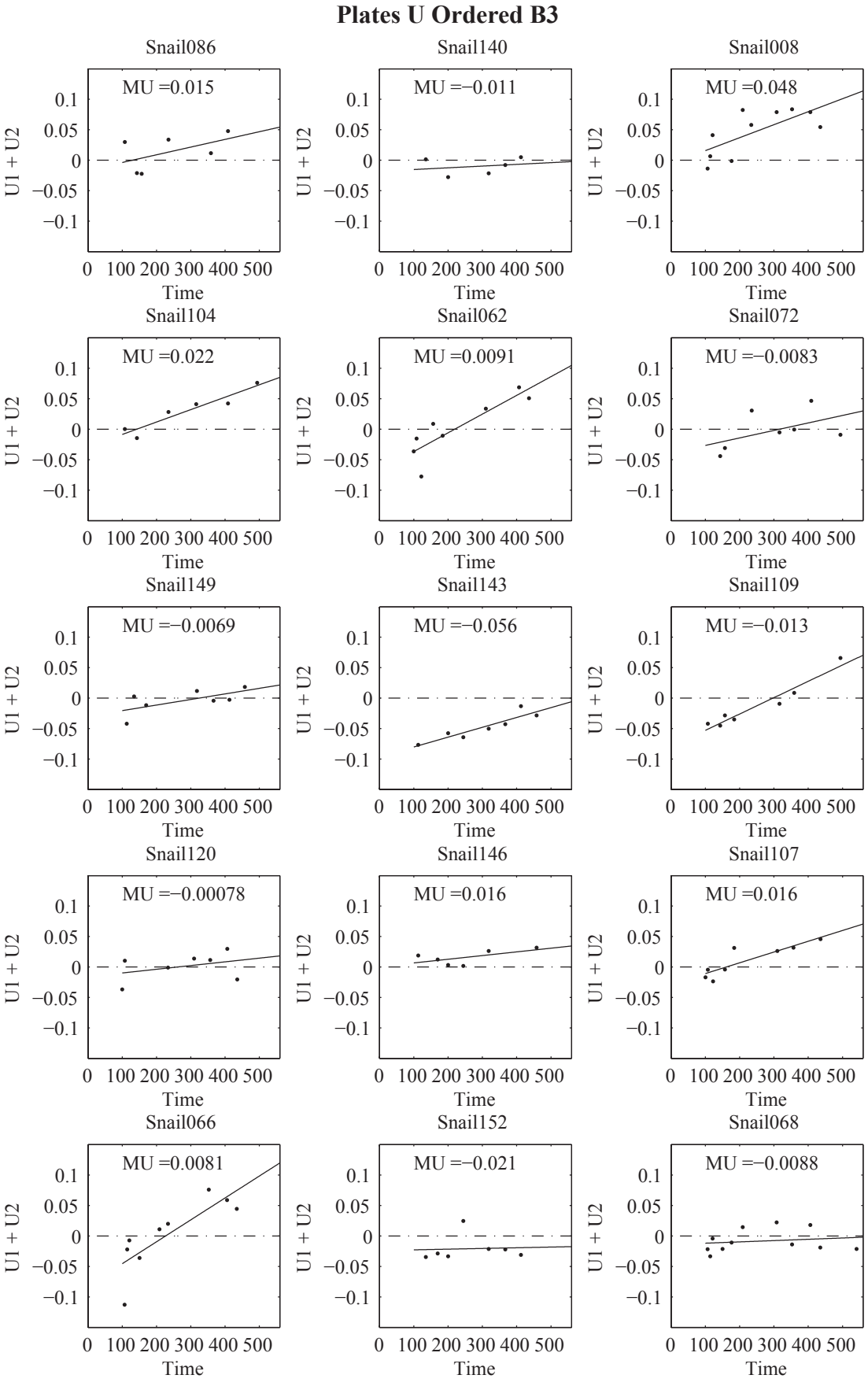

Plates RW3 Ordered B3
Snail038
MRW3 =−0.019
Snail032
MRW3 =0.0022
Snail013
MRW3 =−0.024
Snail030
MRW3 =0.0031
Snail046
MRW3 =−0.0063
Snail119
MRW3 =0.00041
Snail103
MRW3 =0.00021
Snail071
MRW3 =−0.00098
Snail022
MRW3 =−0.0047
Snail048
MRW3 =0.0071
Snail024
MRW3 =−0.013
Snail142
MRW3 =0.0028
Snail026
MRW3 =0.018
Snail058
MRW3 =0.0023
Snail148
MRW3 =0.0098
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

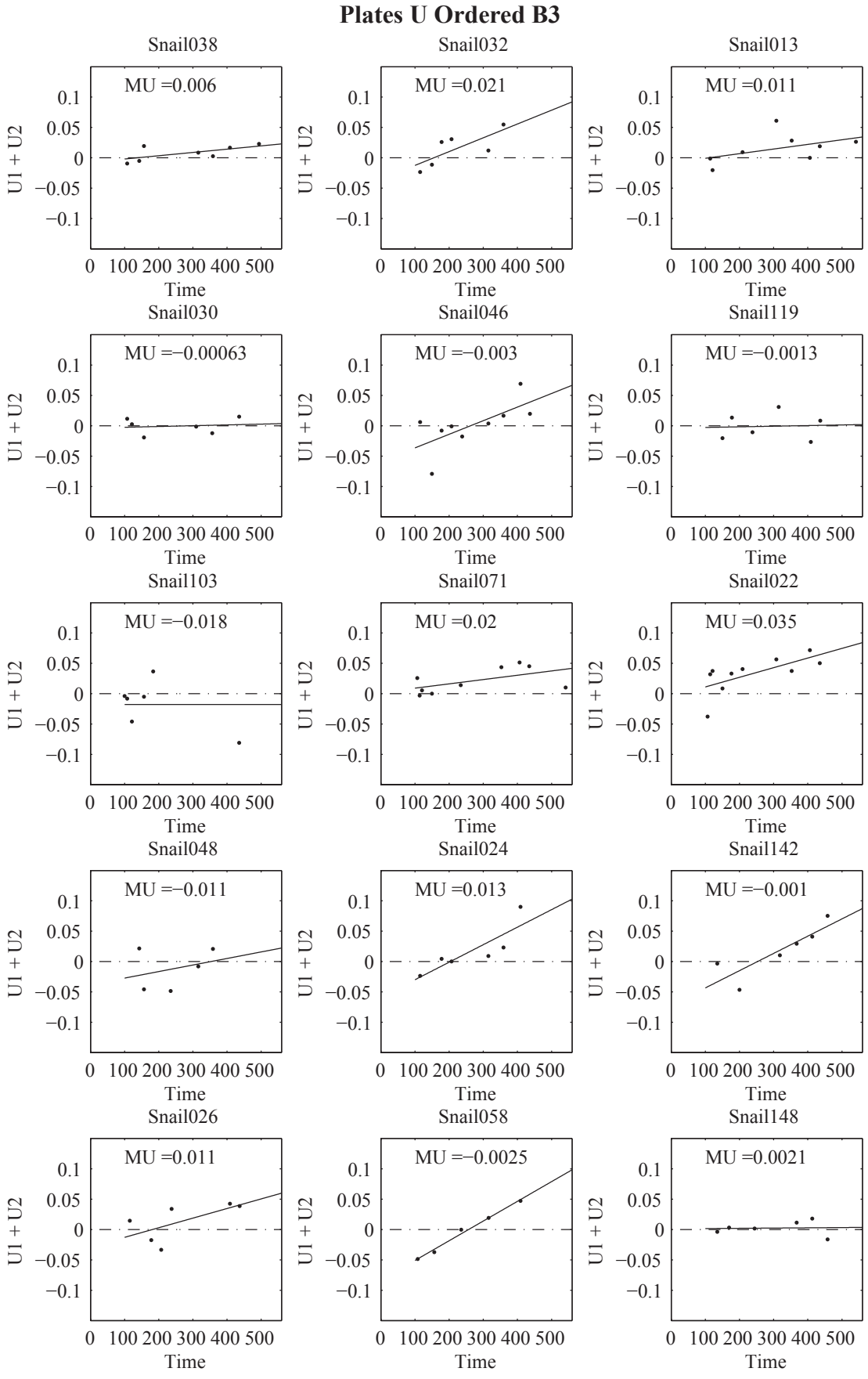

Plates RW3 Ordered B3
Snail041
MRW3 =0.00049
Snail145
MRW3 =0.004
Snail130
MRW3 =0.0057
Snail082
MRW3 =−0.013
Snail055
MRW3 =0.0077
Snail018
MRW3 =0.01
Snail139
MRW3 =0.0088
Snail020
MRW3 =−0.0083
Snail116
MRW3 =−0.003
Snail009
MRW3 =−0.00028
Snail051
MRW3 =0.0039
Snail156
MRW3 =−0.0034
Snail052
MRW3 =0.029
Snail147
MRW3 =−0.0085
Snail098
MRW3 =0.0026
RW3
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

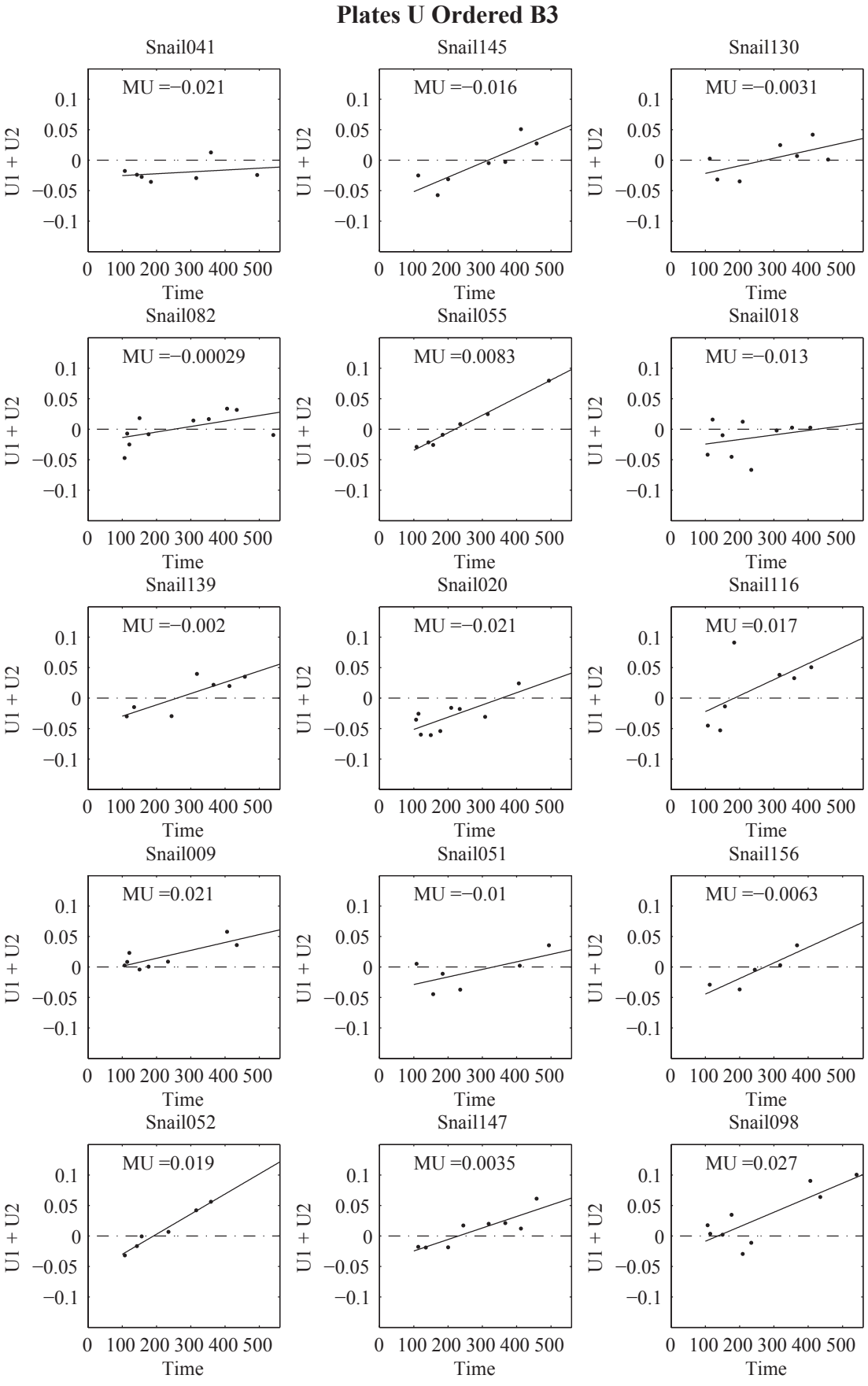

Plates U Ordered B3
Snail201
MU =−0.014
Snail056
MU =0.00099
Snail083
MU =0.0035
Snail112
MU =−0.0042
Snail031
MU =0.023
Snail064
MU =−0.014
Snail136
MU =0.0067
Snail067
MU =−0.032
Snail134
MU =−0.00061
Snail044
MU =0.0054
Snail029
MU =0.0097
Snail007
MU =0.01
Snail006
MU =−0.02
Snail023
MU =0.027
Snail111
MU =0.009
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

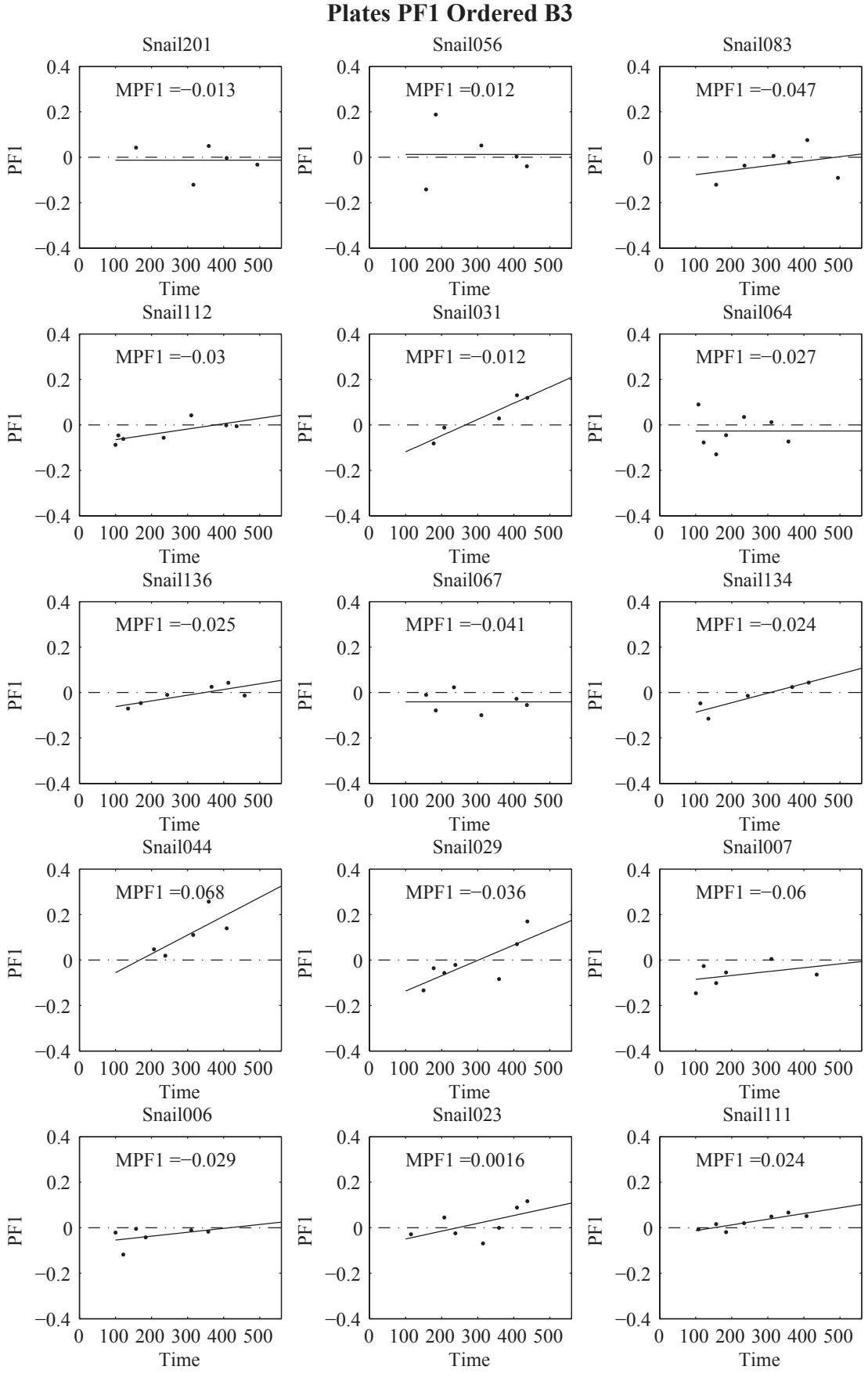

Plates U Ordered B3
Snail065
MU =0.019
Snail047
MU =−0.015
Snail045
MU =−0.007
Snail053
MU =0.022
Snail057
MU =0.011
Snail096
MU =0.0021
Snail088
MU =−0.0032
Snail010
MU =−0.019
Snail061
MU =−0.0073
Snail095
MU =0.0051
Snail036
MU =−0.011
Snail012
MU =−0.023
Snail063
MU =−0.0027
Snail073
MU =−0.0095
Snail144
MU =−0.0095
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

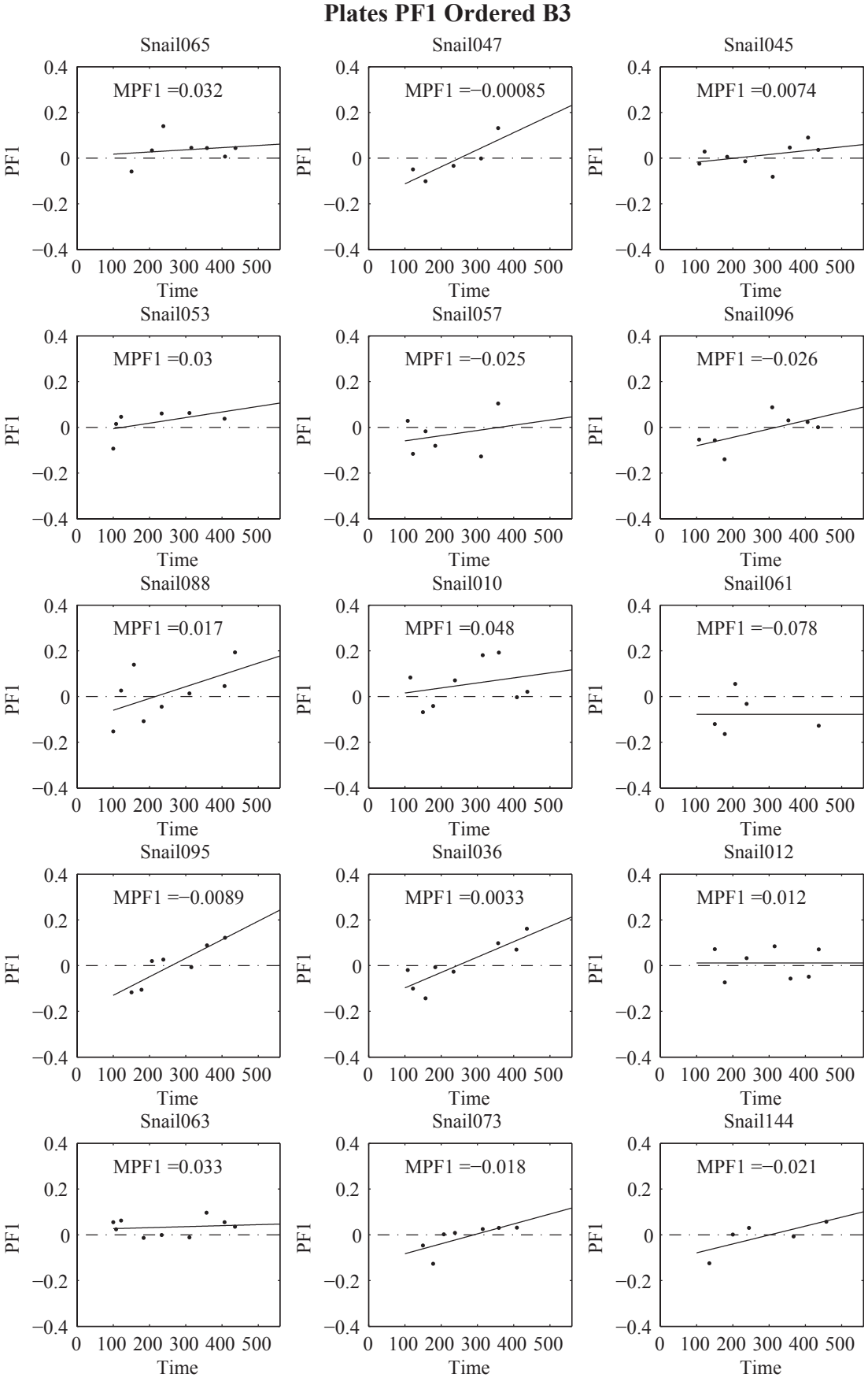

Plates U Ordered B3
Snail153
MU =−0.0036
Snail092
MU =0.0052
Snail021
MU =−0.022
Snail028
MU =0.0058
Snail002
MU =0.018
Snail078
MU =0.033
Snail141
MU =−0.0082
Snail133
MU =0.02
Snail035
MU =−0.00049
Snail090
MU =−0.0068
Snail150
MU =−0.0059
Snail034
MU =0.0052
Snail043
MU =−0.023
Snail131
MU =−0.01
Snail100
MU =0.0058
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

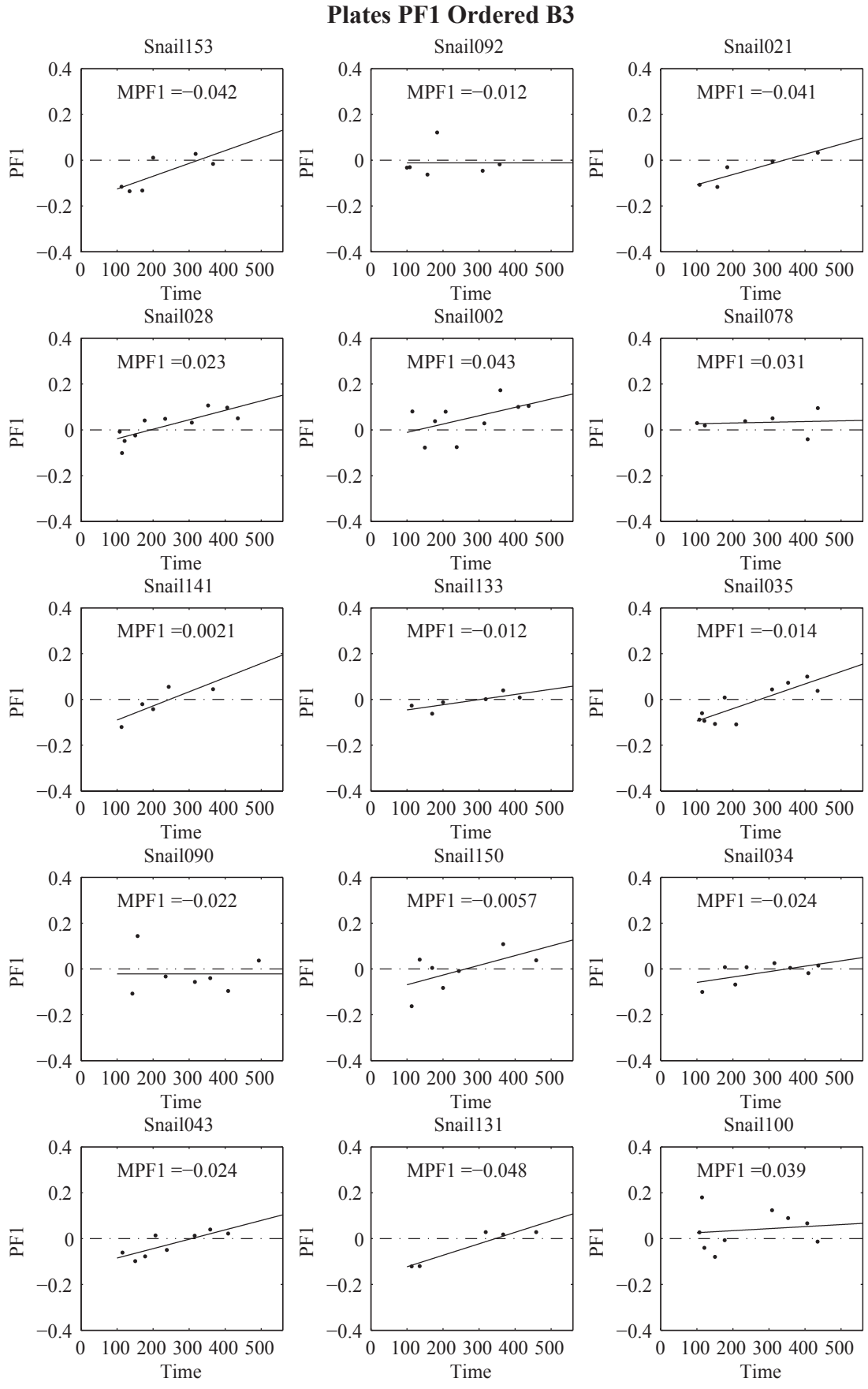

Plates U Ordered B3
Snail086
MU =0.015
Snail140
MU =−0.011
Snail008
MU =0.048
Snail104
MU =0.022
Snail062
MU =0.0091
Snail072
MU =−0.0083
Snail149
MU =−0.0069
Snail143
MU =−0.056
Snail109
MU =−0.013
Snail120
MU =−0.00078
Snail146
MU =0.016
Snail107
MU =0.016
Snail066
MU =0.0081
Snail152
MU =−0.021
Snail068
MU =−0.0088
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

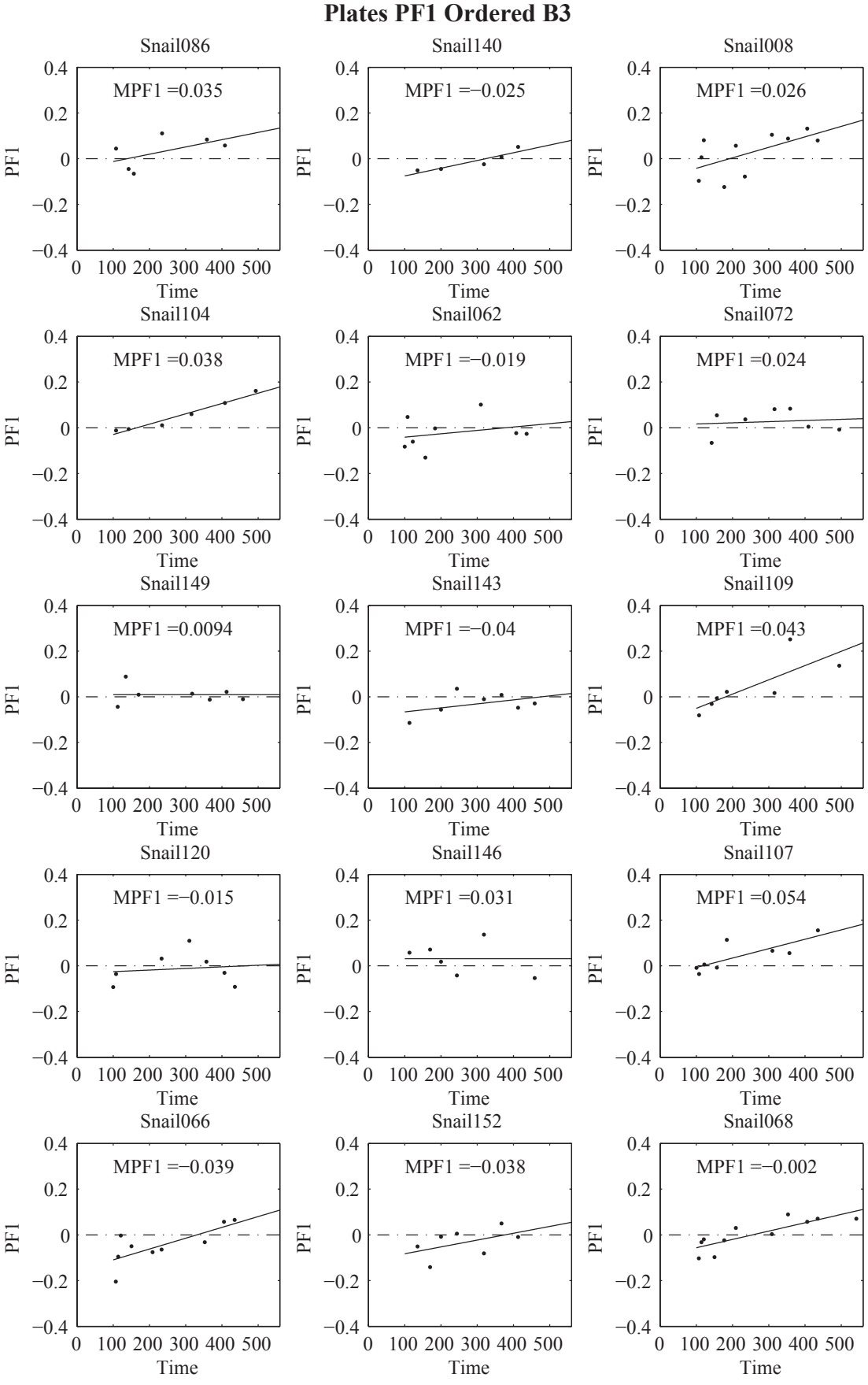

Plates U Ordered B3
Snail038
MU =0.006
Snail032
MU =0.021
Snail013
MU =0.011
Snail030
MU =−0.00063
Snail046
MU =−0.003
Snail119
MU =−0.0013
Snail103
MU =−0.018
Snail071
MU =0.02
Snail022
MU =0.035
Snail048
MU =−0.011
Snail024
MU =0.013
Snail142
MU =−0.001
Snail026
MU =0.011
Snail058
MU =−0.0025
Snail148
MU =0.0021
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

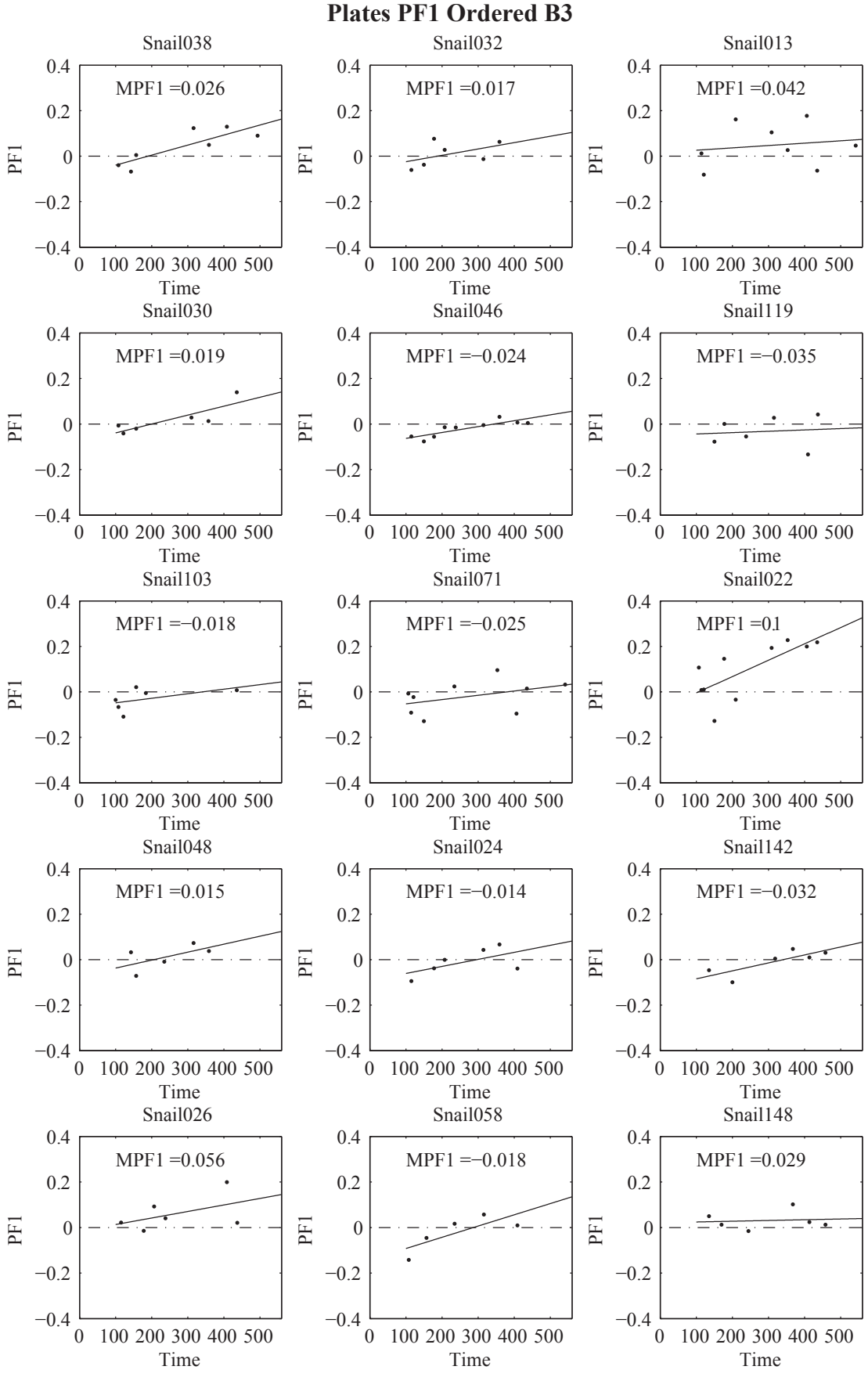

Plates U Ordered B3
Snail041
MU =−0.021
Snail145
MU =−0.016
Snail130
MU =−0.0031
Snail082
MU =−0.00029
Snail055
MU =0.0083
Snail018
MU =−0.013
Snail139
MU =−0.002
Snail020
MU =−0.021
Snail116
MU =0.017
Snail009
MU =0.021
Snail051
MU =−0.01
Snail156
MU =−0.0063
Snail052
MU =0.019
Snail147
MU =0.0035
Snail098
MU =0.027
U1 + U2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

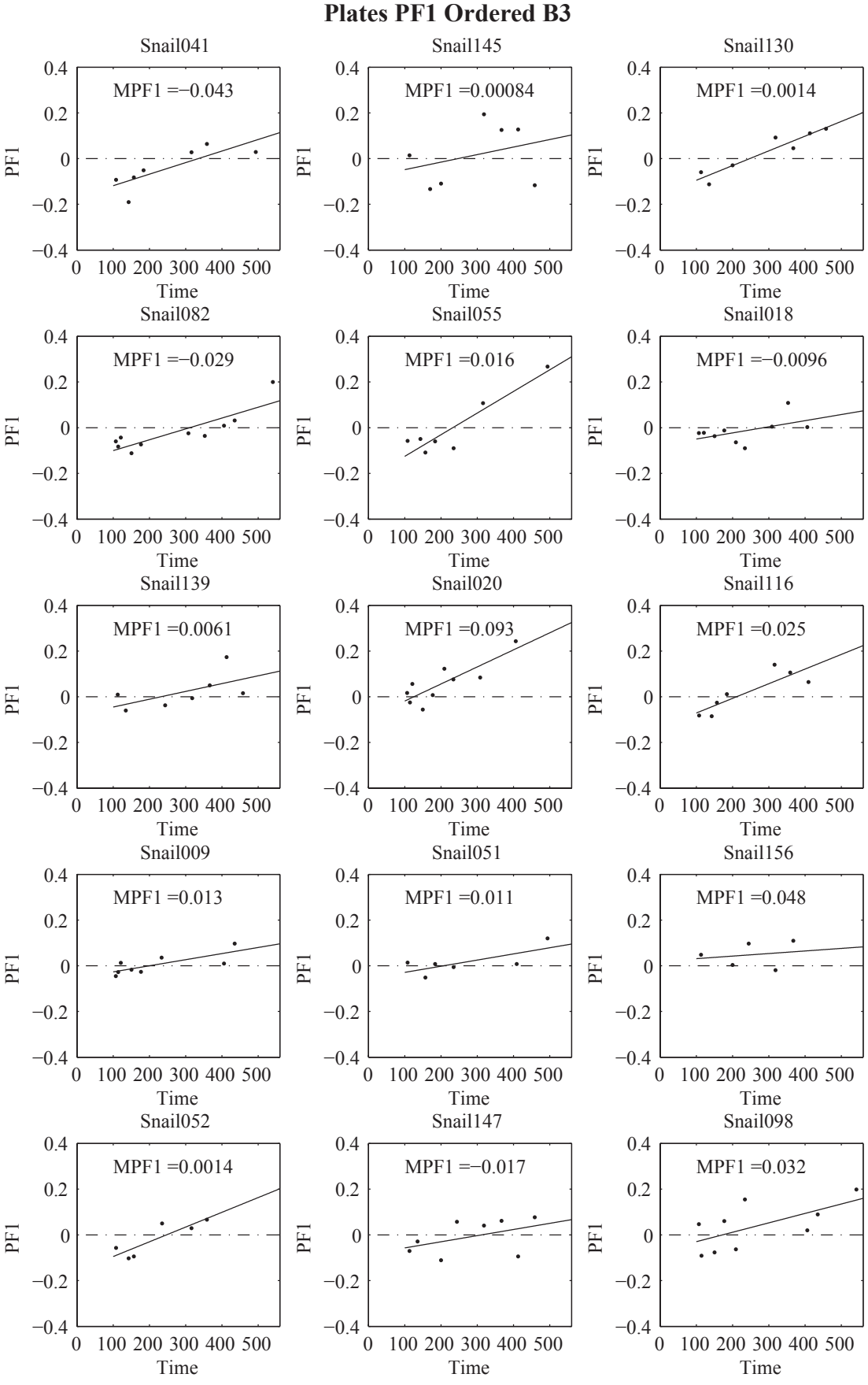

Plates PF1 Ordered B3
Snail201
MPF1 =−0.013
Snail056
MPF1 =0.012
Snail083
MPF1 =−0.047
Snail112
MPF1 =−0.03
Snail031
MPF1 =−0.012
Snail064
MPF1 =−0.027
Snail136
MPF1 =−0.025
Snail067
MPF1 =−0.041
Snail134
MPF1 =−0.024
Snail044
MPF1 =0.068
Snail029
MPF1 =−0.036
Snail007
MPF1 =−0.06
Snail006
MPF1 =−0.029
Snail023
MPF1 =0.0016
Snail111
MPF1 =0.024
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

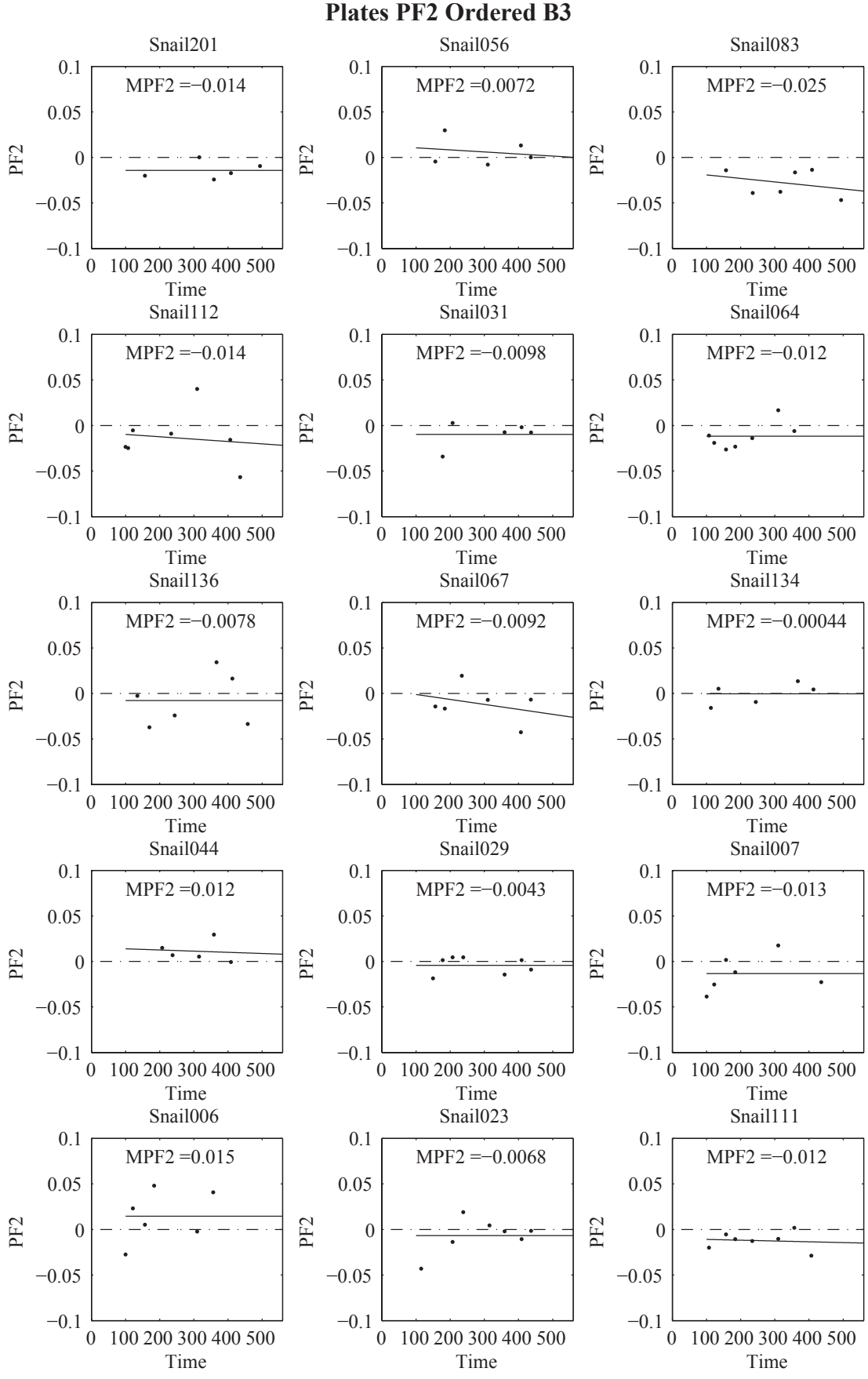

Plates PF1 Ordered B3
Snail065
MPF1 =0.032
Snail047
MPF1 =−0.00085
Snail045
MPF1 =0.0074
Snail053
MPF1 =0.03
Snail057
MPF1 =−0.025
Snail096
MPF1 =−0.026
Snail088
MPF1 =0.017
Snail010
MPF1 =0.048
Snail061
MPF1 =−0.078
Snail095
MPF1 =−0.0089
Snail036
MPF1 =0.0033
Snail012
MPF1 =0.012
Snail063
MPF1 =0.033
Snail073
MPF1 =−0.018
Snail144
MPF1 =−0.021
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

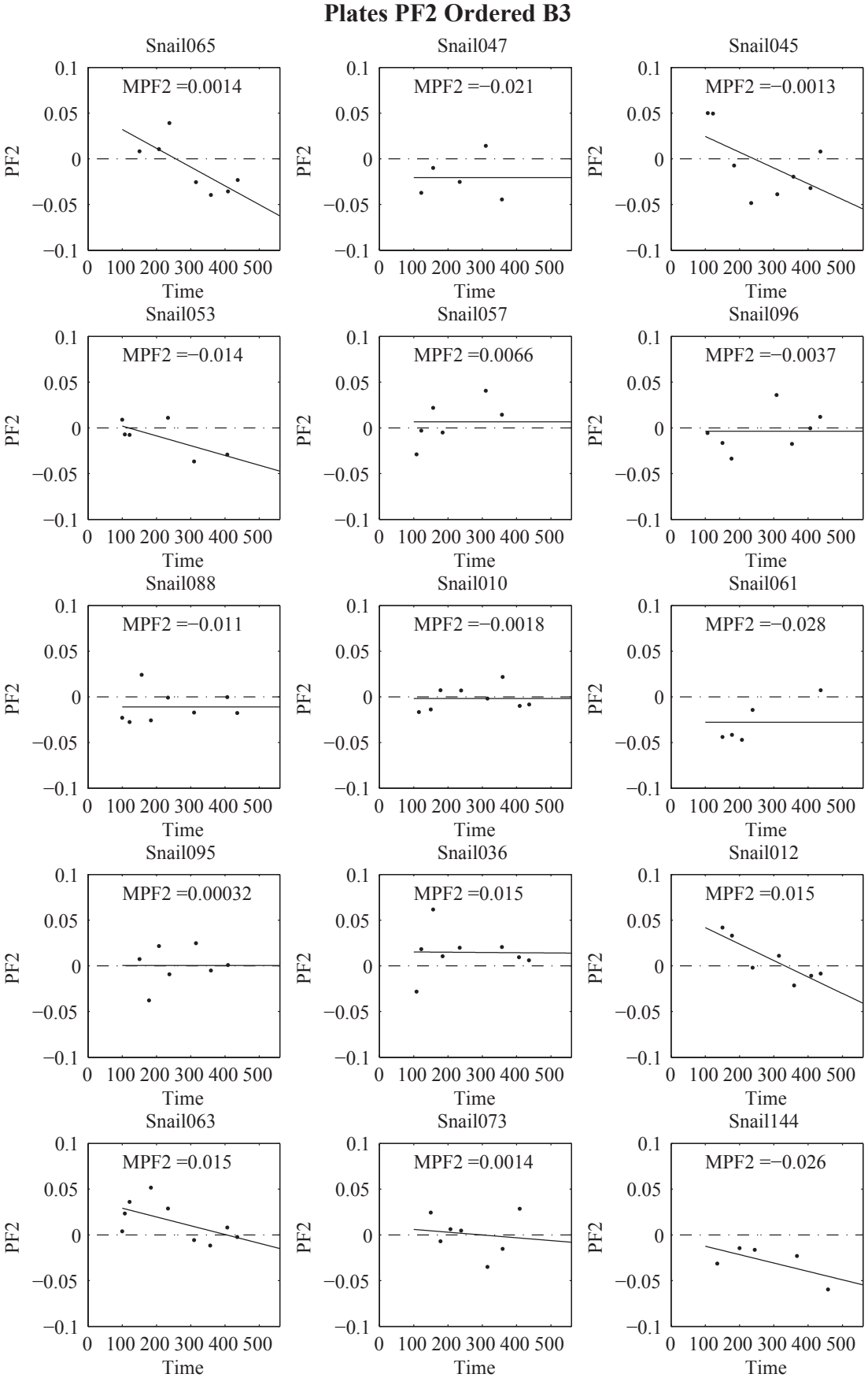

Plates PF1 Ordered B3
Snail153
MPF1 =−0.042
Snail092
MPF1 =−0.012
Snail021
MPF1 =−0.041
Snail028
MPF1 =0.023
Snail002
MPF1 =0.043
Snail078
MPF1 =0.031
Snail141
MPF1 =0.0021
Snail133
MPF1 =−0.012
Snail035
MPF1 =−0.014
Snail090
MPF1 =−0.022
Snail150
MPF1 =−0.0057
Snail034
MPF1 =−0.024
Snail043
MPF1 =−0.024
Snail131
MPF1 =−0.048
Snail100
MPF1 =0.039
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

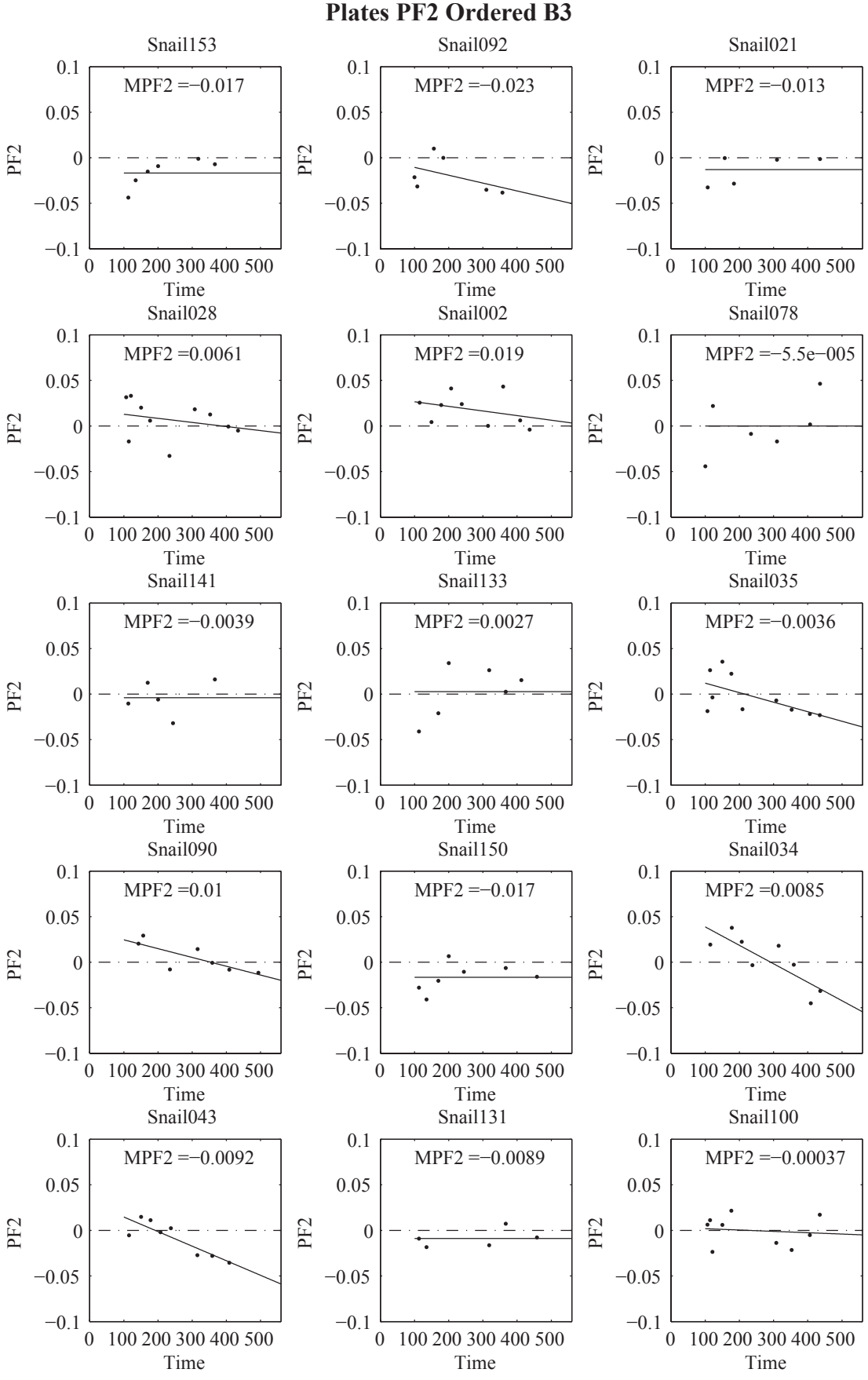

Plates PF1 Ordered B3
Snail086
MPF1 =0.035
Snail140
MPF1 =−0.025
Snail008
MPF1 =0.026
Snail104
MPF1 =0.038
Snail062
MPF1 =−0.019
Snail072
MPF1 =0.024
Snail149
MPF1 =0.0094
Snail143
MPF1 =−0.04
Snail109
MPF1 =0.043
Snail120
MPF1 =−0.015
Snail146
MPF1 =0.031
Snail107
MPF1 =0.054
Snail066
MPF1 =−0.039
Snail152
MPF1 =−0.038
Snail068
MPF1 =−0.002
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

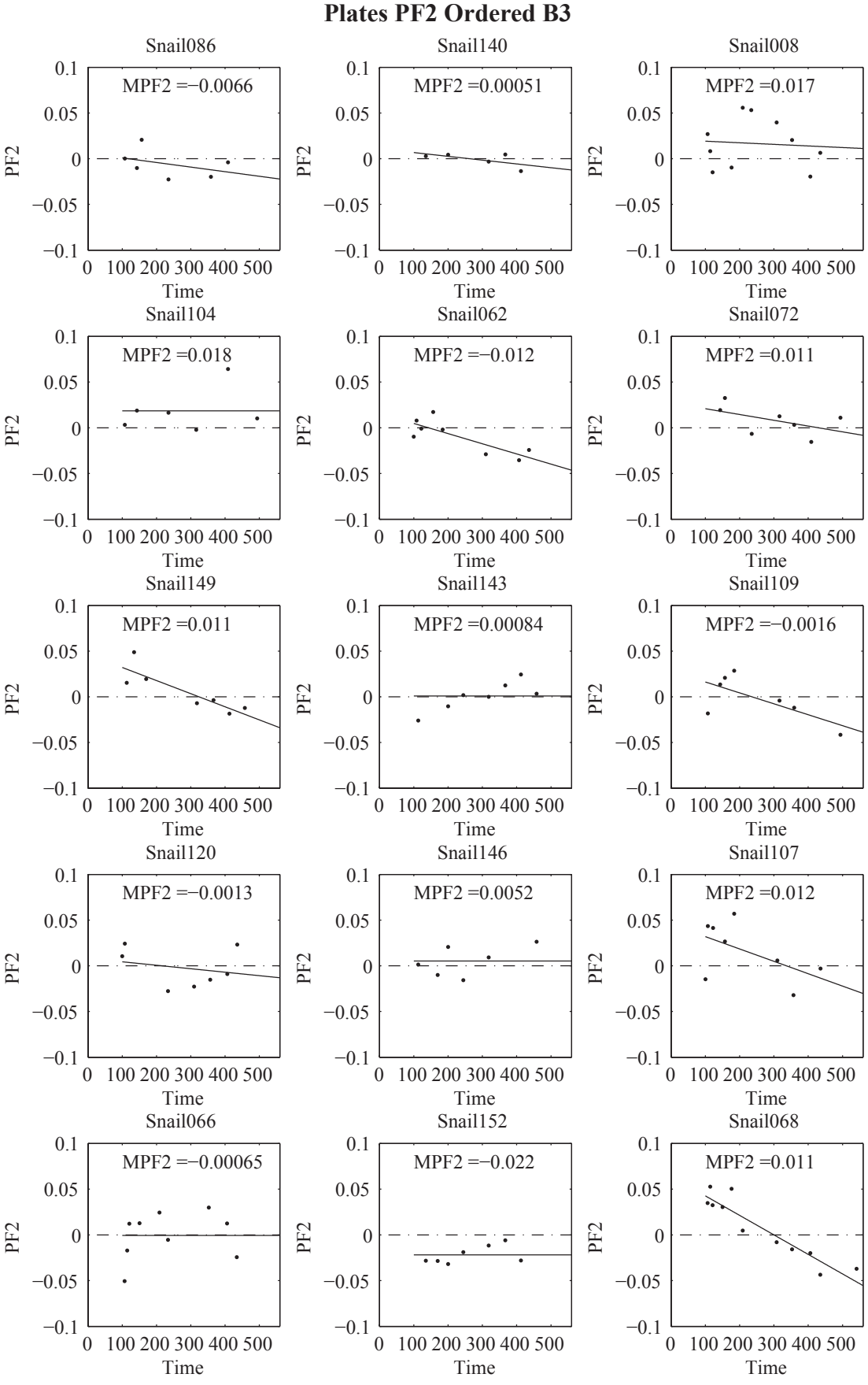

Plates PF1 Ordered B3
Snail038
MPF1 =0.026
Snail032
MPF1 =0.017
Snail013
MPF1 =0.042
Snail030
MPF1 =0.019
Snail046
MPF1 =−0.024
Snail119
MPF1 =−0.035
Snail103
MPF1 =−0.018
Snail071
MPF1 =−0.025
Snail022
MPF1 =01
Snail048
MPF1 =0.015
Snail024
MPF1 =−0.014
Snail142
MPF1 =−0.032
Snail026
MPF1 =0.056
Snail058
MPF1 =−0.018
Snail148
MPF1 =0.029
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

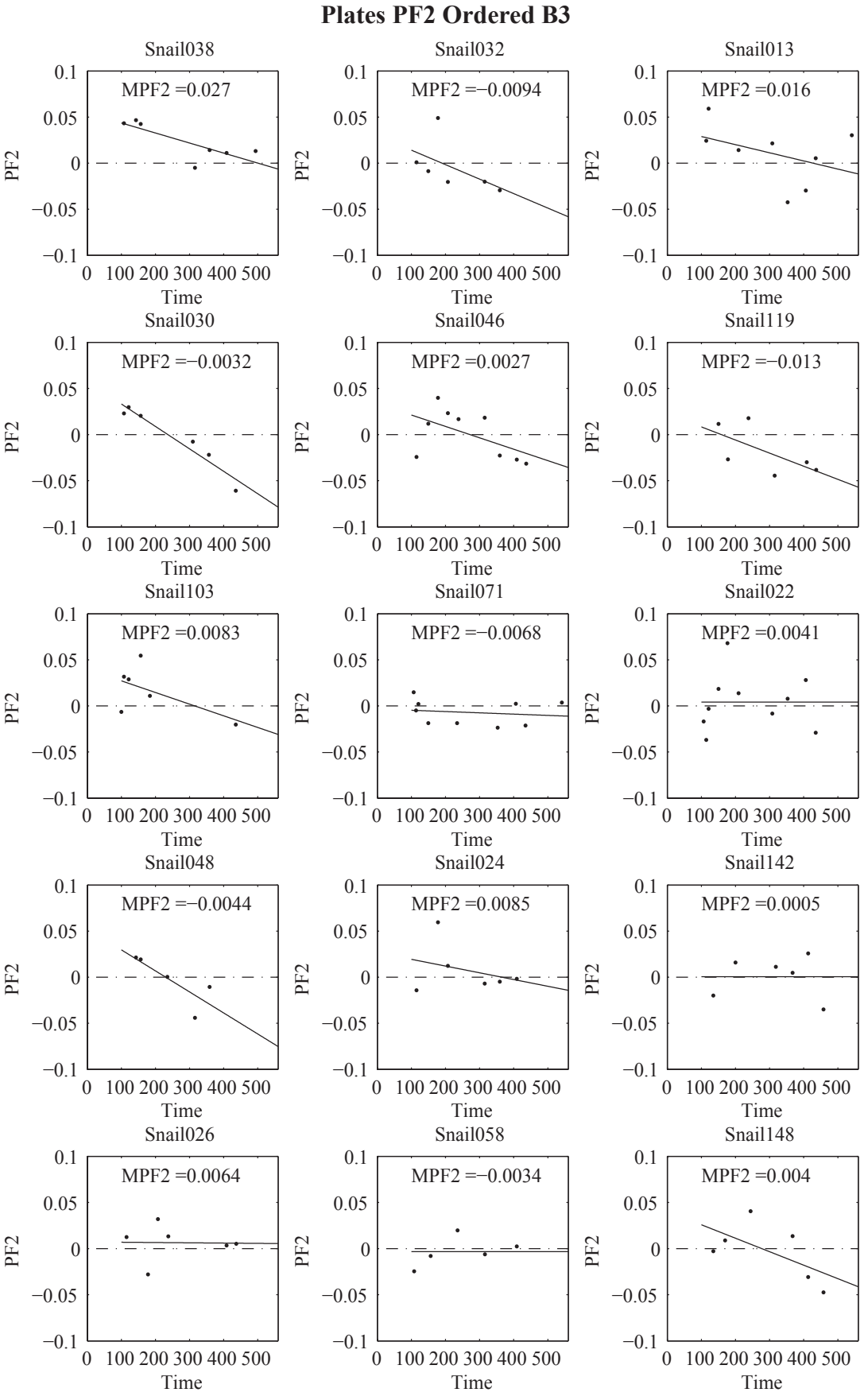

Plates PF1 Ordered B3
Snail041
MPF1 =−0.043
Snail145
MPF1 =0.00084
Snail130
MPF1 =0.0014
Snail082
MPF1 =−0.029
Snail055
MPF1 =0.016
Snail018
MPF1 =−0.0096
Snail139
MPF1 =0.0061
Snail020
MPF1 =0.093
Snail116
MPF1 =0.025
Snail009
MPF1 =0.013
Snail051
MPF1 =0.011
Snail156
MPF1 =0.048
Snail052
MPF1 =0.0014
Snail147
MPF1 =−0.017
Snail098
MPF1 =0.032
PF1
Time
0.4
0.2
0
−0.2
−0.4
0 100 200 300 400 500

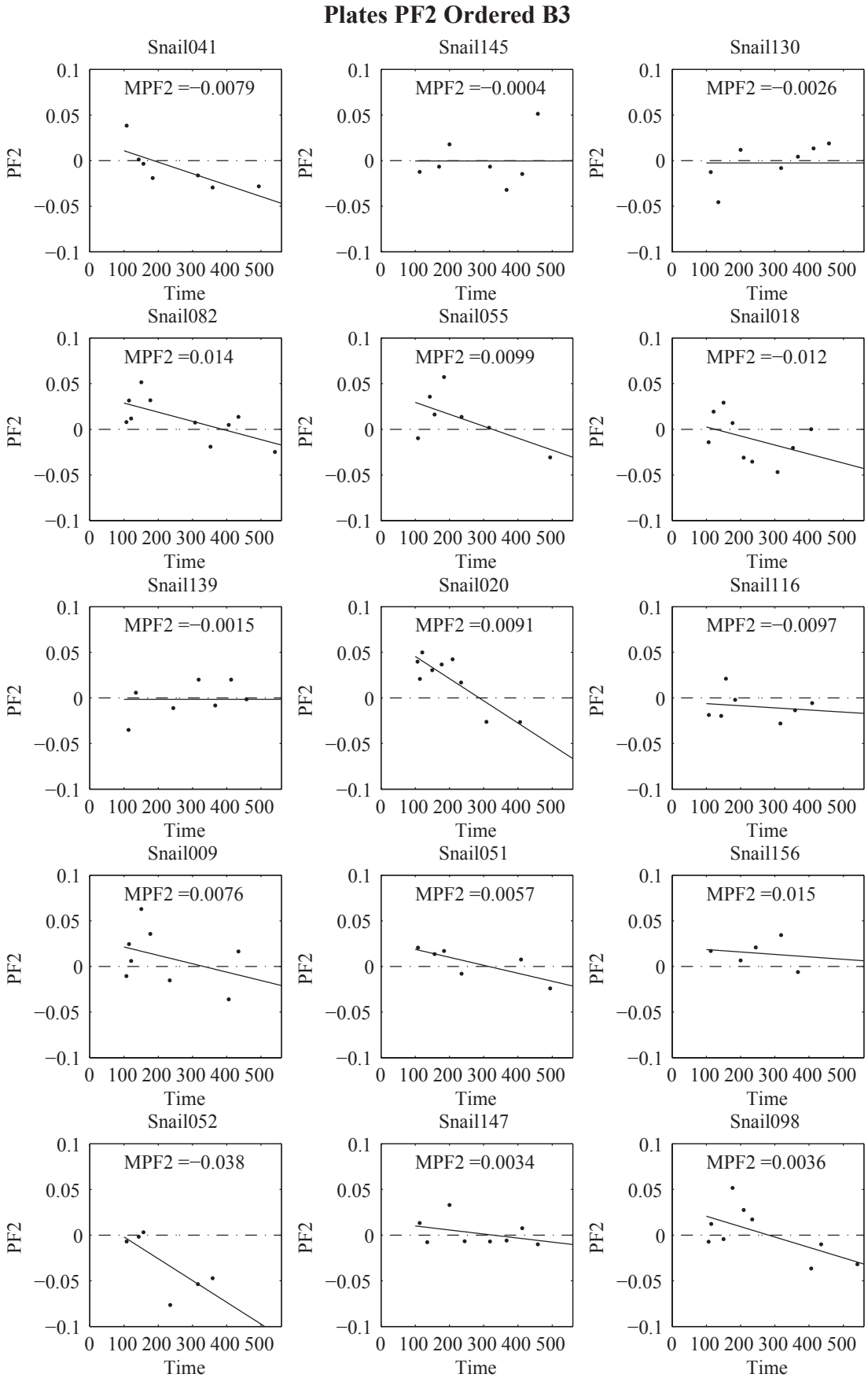

Plates PF2 Ordered B3
Snail201
MPF2 =−0.014
Snail056
MPF2 =0.0072
Snail083
MPF2 =−0.025
Snail112
MPF2 =−0.014
Snail031
MPF2 =−0.0098
Snail064
MPF2 =−0.012
Snail136
MPF2 =−0.0078
Snail067
MPF2 =−0.0092
Snail134
MPF2 =−0.00044
Snail044
MPF2 =0.012
Snail029
MPF2 =−0.0043
Snail007
MPF2 =−0.013
Snail006
MPF2 =0.015
Snail023
MPF2 =−0.0068
Snail111
MPF2 =−0.012
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

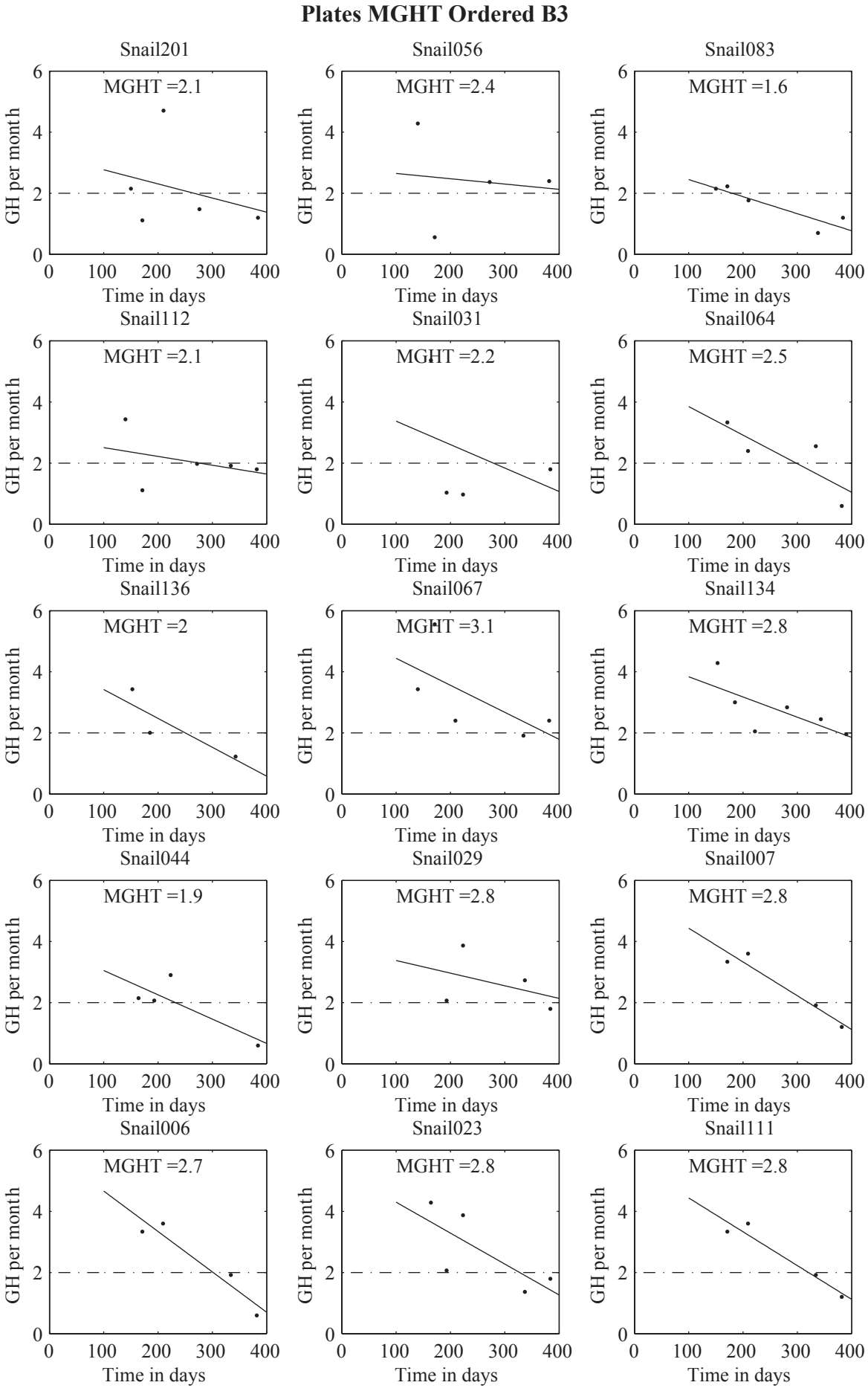

Plates PF2 Ordered B3
Snail065
MPF2 =0.0014
Snail047
MPF2 =−0.021
Snail045
MPF2 =−0.0013
Snail053
MPF2 =−0.014
Snail057
MPF2 =0.0066
Snail096
MPF2 =−0.0037
Snail088
MPF2 =−0.011
Snail010
MPF2 =−0.0018
Snail061
MPF2 =−0.028
Snail095
MPF2 =0.00032
Snail036
MPF2 =0.015
Snail012
MPF2 =0.015
Snail063
MPF2 =0.015
Snail073
MPF2 =0.0014
Snail144
MPF2 =−0.026
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

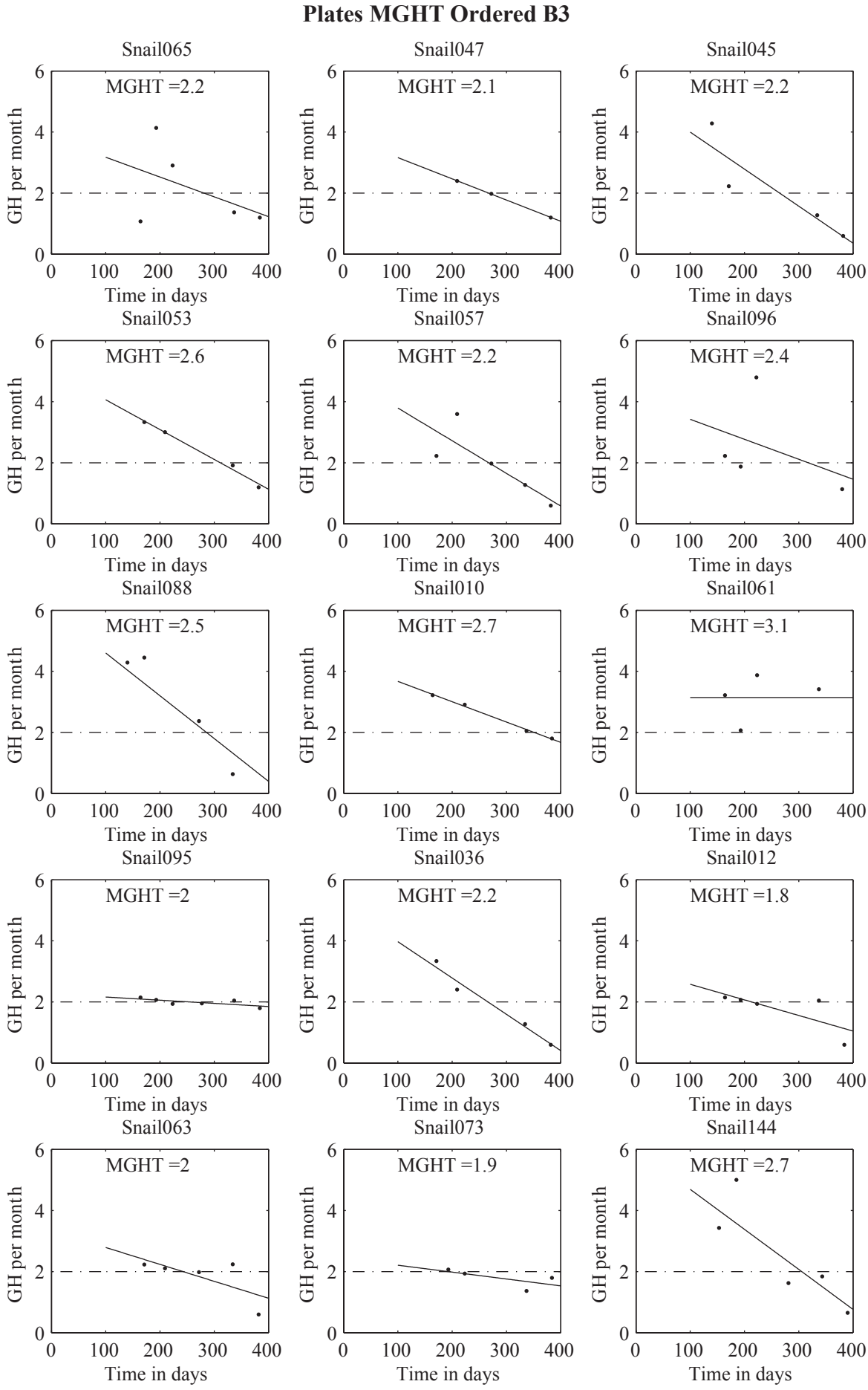

Plates PF2 Ordered B3
Snail153
MPF2 =−0.017
Snail092
MPF2 =−0.023
Snail021
MPF2 =−0.013
Snail028
MPF2 =0.0061
Snail002
MPF2 =0.019
Snail078
MPF2 =−5.5e−005
Snail141
MPF2 =−0.0039
Snail133
MPF2 =0.0027
Snail035
MPF2 =−0.0036
Snail090
MPF2 =0.01
Snail150
MPF2 =−0.017
Snail034
MPF2 =0.0085
Snail043
MPF2 =−0.0092
Snail131
MPF2 =−0.0089
Snail100
MPF2 =−0.00037
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

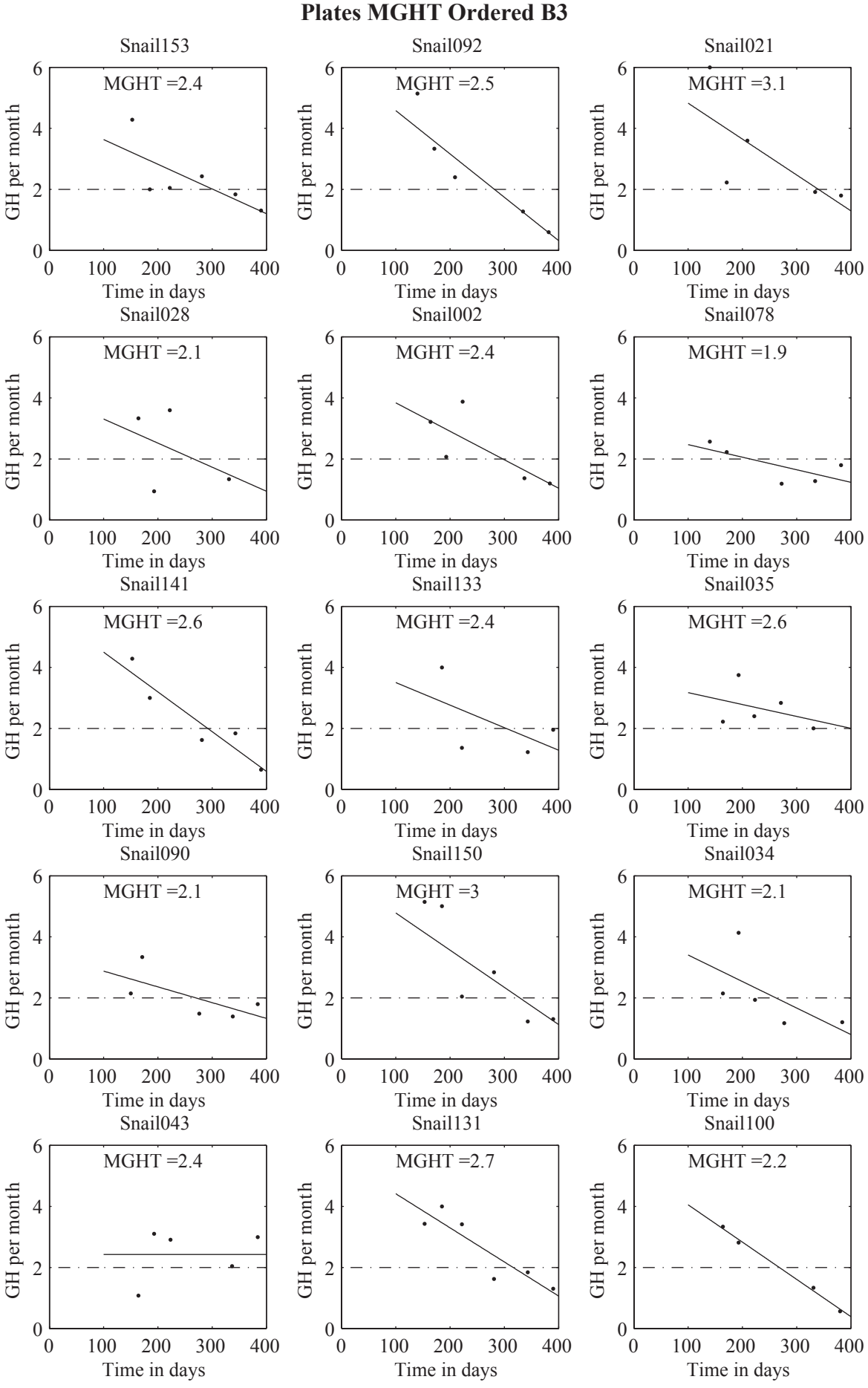

Plates PF2 Ordered B3
Snail086
MPF2 =−0.0066
Snail140
MPF2 =0.00051
Snail008
MPF2 =0.017
Snail104
MPF2 =0.018
Snail062
MPF2 =−0.012
Snail072
MPF2 =0.011
Snail149
MPF2 =0.011
Snail143
MPF2 =0.00084
Snail109
MPF2 =−0.0016
Snail120
MPF2 =−0.0013
Snail146
MPF2 =0.0052
Snail107
MPF2 =0.012
Snail066
MPF2 =−0.00065
Snail152
MPF2 =−0.022
Snail068
MPF2 =0.011
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

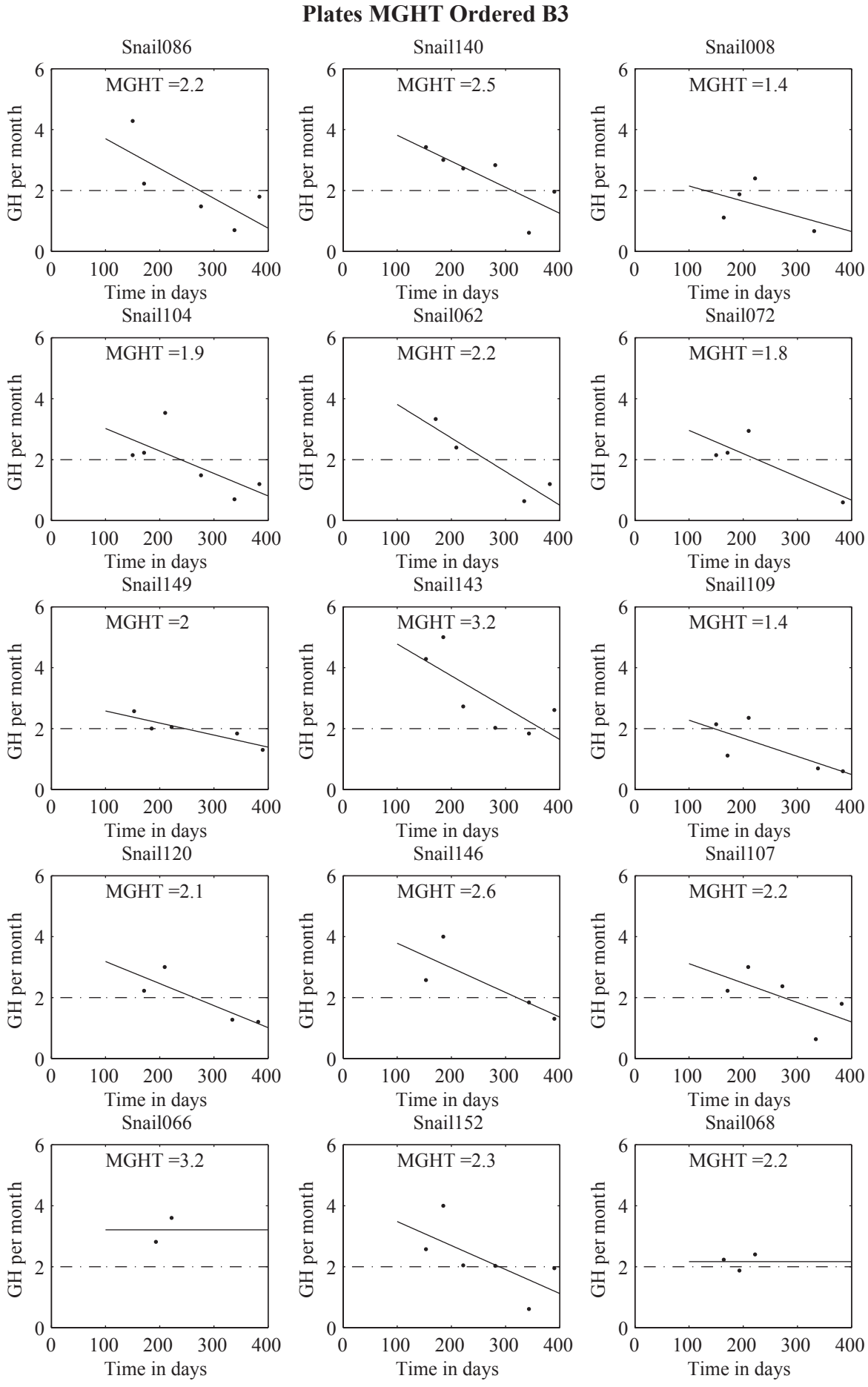

Plates PF2 Ordered B3
Snail038
MPF2 =0.027
Snail032
MPF2 =−0.0094
Snail013
MPF2 =0.016
Snail030
MPF2 =−0.0032
Snail046
MPF2 =0.0027
Snail119
MPF2 =−0.013
Snail103
MPF2 =0.0083
Snail071
MPF2 =−0.0068
Snail022
MPF2 =0.0041
Snail048
MPF2 =−0.0044
Snail024
MPF2 =0.0085
Snail142
MPF2 =0.0005
Snail026
MPF2 =0.0064
Snail058
MPF2 =−0.0034
Snail148
MPF2 =0.004
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

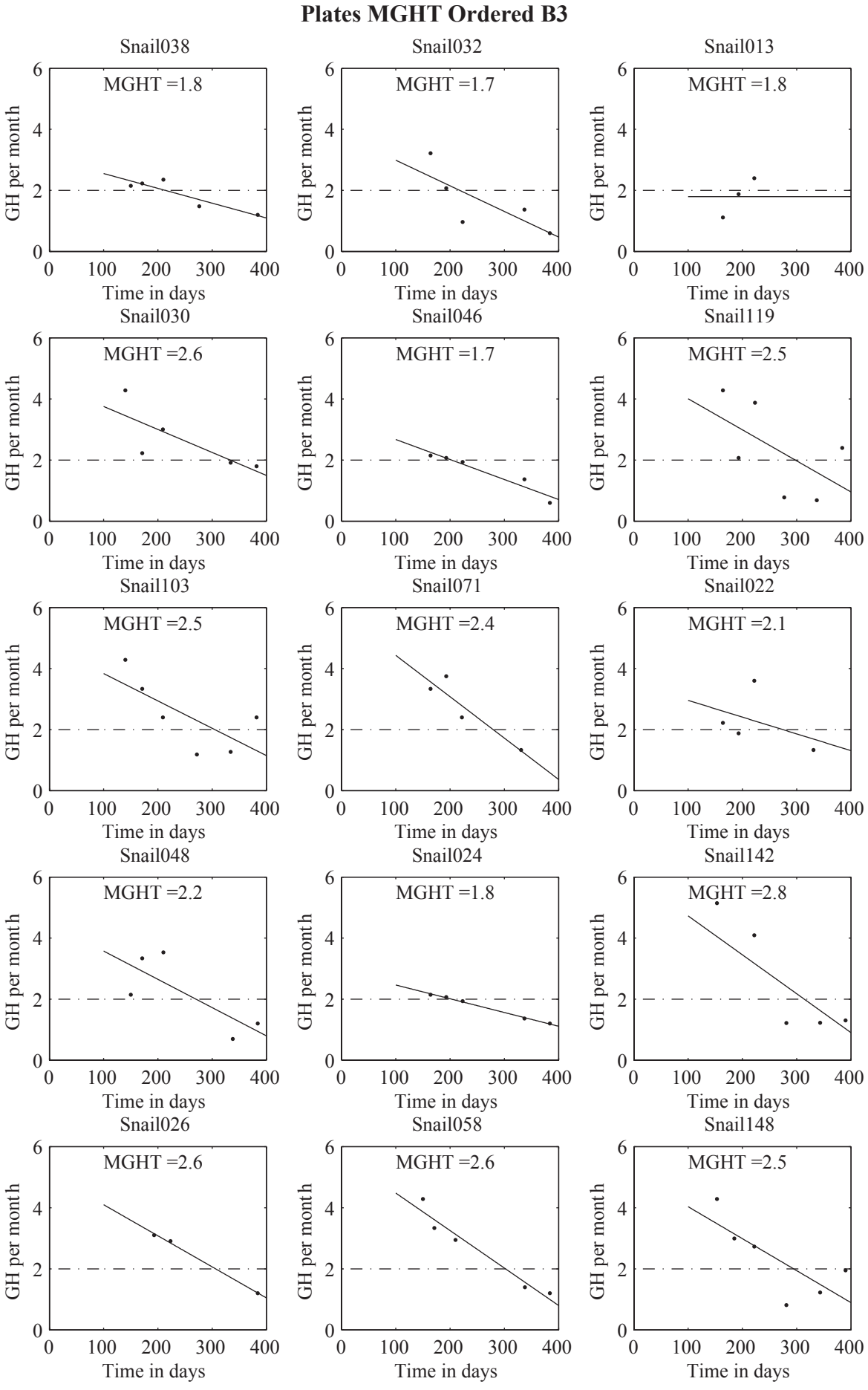

Plates PF2 Ordered B3
Snail041
MPF2 =−0.0079
Snail145
MPF2 =−0.0004
Snail130
MPF2 =−0.0026
Snail082
MPF2 =0.014
Snail055
MPF2 =0.0099
Snail018
MPF2 =−0.012
Snail139
MPF2 =−0.0015
Snail020
MPF2 =0.0091
Snail116
MPF2 =−0.0097
Snail009
MPF2 =0.0076
Snail051
MPF2 =0.0057
Snail156
MPF2 =0.015
Snail052
MPF2 =−0.038
Snail147
MPF2 =0.0034
Snail098
MPF2 =0.0036
PF2
Time
0.1
0.05
0
−0.05
−0.1
0 100 200 300 400 500

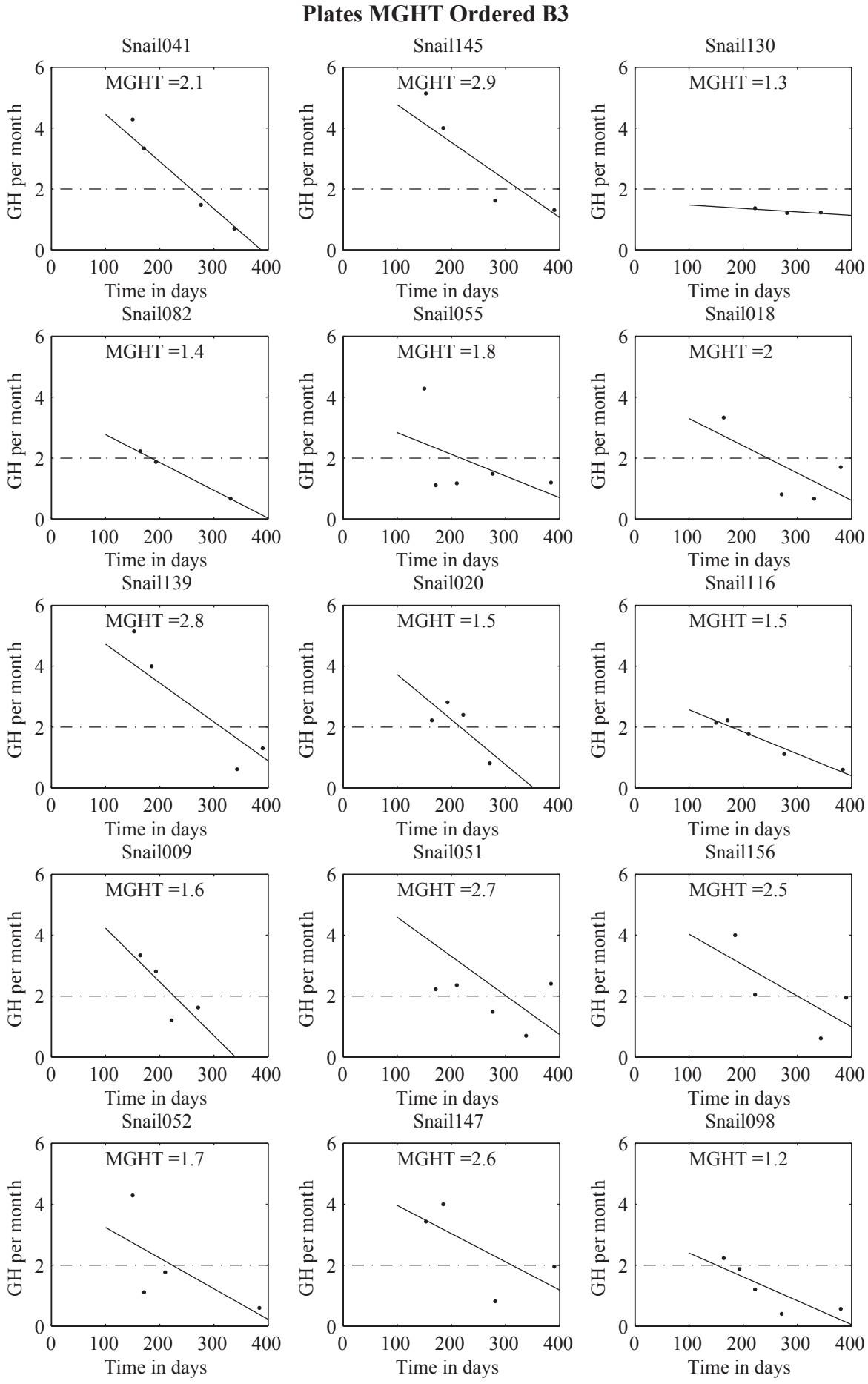

Plates MGHT Ordered B3
Snail201
MGHT =2.1
Snail056
MGHT =2.4
Snail083
MGHT =1.6
Snail112
MGHT =2.1
Snail031
MGHT =2.2
Snail064
MGHT =2.5
Snail136
MGHT =2
Snail067
MGHT =3.1
Snail134
MGHT =2.8
Snail044
MGHT =1.9
Snail029
MGHT =2.8
Snail007
MGHT =2.8
Snail006
MGHT =2.7
Snail023
MGHT =2.8
Snail111
MGHT =2.8
GH per month
Time in days
0
2
4
6
0
100
200
300
400


Plates MGHT Ordered B3
Snail065
MGHT =2.2
Snail047
MGHT =2.1
Snail045
MGHT =2.2
Snail053
MGHT =2.6
Snail057
MGHT =2.2
Snail096
MGHT =2.4
Snail088
MGHT =2.5
Snail010
MGHT =2.7
Snail061
MGHT =3.1
Snail095
MGHT =2
Snail036
MGHT =2.2
Snail012
MGHT =1.8
Snail063
MGHT =2
Snail073
MGHT =1.9
Snail144
MGHT =2.7
GH per month
Time in days
0
2
4
6
0
100
200
300
400


Plates MGHT Ordered B3
Snail153
MGHT =2.4
Snail092
MGHT =2.5
Snail021
MGHT =3.1
Snail028
MGHT =2.1
Snail002
MGHT =2.4
Snail078
MGHT =1.9
Snail141
MGHT =2.6
Snail133
MGHT =2.4
Snail035
MGHT =2.6
Snail090
MGHT =2.1
Snail150
MGHT =3
Snail034
MGHT =2.1
Snail043
MGHT =2.4
Snail131
MGHT =2.7
Snail100
MGHT =2.2
GH per month
Time in days
0
2
4
6
0
100
200
300
400


Plates MGHT Ordered B3
Snail086
MGHT =2.2
Snail140
MGHT =2.5
Snail008
MGHT =1.4
Snail104
MGHT =1.9
Snail062
MGHT =2.2
Snail072
MGHT =1.8
Snail149
MGHT =2
Snail143
MGHT =3.2
Snail109
MGHT =1.4
Snail120
MGHT =2.1
Snail146
MGHT =2.6
Snail107
MGHT =2.2
Snail066
MGHT =3.2
Snail152
MGHT =2.3
Snail068
MGHT =2.2
GH per month
Time in days
0
2
4
6
0
100
200
300
400


Plates MGHT Ordered B3
Snail038
MGHT =1.8
Snail032
MGHT =1.7
Snail013
MGHT =1.8
Snail030
MGHT =2.6
Snail046
MGHT =1.7
Snail119
MGHT =2.5
Snail103
MGHT =2.5
Snail071
MGHT =2.4
Snail022
MGHT =2.1
Snail048
MGHT =2.2
Snail024
MGHT =1.8
Snail142
MGHT =2.8
Snail026
MGHT =2.6
Snail058
MGHT =2.6
Snail148
MGHT =2.5
GH per month
Time in days
0
2
4
6
0
100
200
300
400


Plates MGHT Ordered B3
Snail041
MGHT =2.1
Snail145
MGHT =2.9
Snail130
MGHT =1.3
Snail082
MGHT =1.4
Snail055
MGHT =1.8
Snail018
MGHT =2
Snail139
MGHT =2.8
Snail020
MGHT =1.5
Snail116
MGHT =1.5
Snail009
MGHT =1.6
Snail051
MGHT =2.7
Snail156
MGHT =2.5
Snail052
MGHT =1.7
Snail147
MGHT =2.6
Snail098
MGHT =1.2
GH per month
Time in days
0
2
4
6
0
100
200
300
400

CONCLUSIONS

In the first part of this dissertation, **chapter 1** laid emphasis on the 'developmental side' of evo-devo, while **chapter 2** underscored the 'evolutionary side' of evo-devo. These two chapters tried to draw the connections between different subfields: developmental genetics, cell biology, medicine, computational biology, tissue engineering and Neo-Darwinism. They discussed how generic models could inform us about the generation and evolution of structures of particular size and shape.

The second part of this dissertation dealt with what Waddington referred to as *'secondary morphogenesis'*: the relationship between growth and form. **Chapters 3** & **4** touched upon the question of what kind of morphological variation is expected given some basic rules of growth. Relying on the knowledge gained in chapters 3 & 4, **chapter 5** referred to what kind of rules could generate some of the observed patterns of covariation between shell characters.

From chapters 3 to 5, we progressively moved from patterns of ontogenetic variation to patterns of phenotypic variation in populations, while trying to keep the link between the two. Simultaneously, we progressively shifted from theory to experiment to finally turn back to theory at the end of chapter 5. **Chapter 3** discussed the implications regarding the (much forgotten) relationship between growth rates and allometry in general and molluscs in particular. **Chapter 4** highlighted how different sub-data sets could lead to different interpretations of the same phenomenon at the level of populations. It also pointed out the impossibility, both empirically and (even more crucially) theoretically, of determining how much phenotypic variation is due to variation in 'genotype' and how much is due to variation in the 'environment'. Links with ecology were drawn here, although they remained in a preliminary stage. Besides describing, in **chapter 5**, the individual patterns of ontogenetic variation in shell growth and shell shape in a population of *Hexaplex (Trunculariopsis) trunculus* Linné 1758, the growth vector model, developed in chapters 3 & 4, suggested that variation in growth rhythm was critical to generate some patterns of covariation between shell characters. This chapter highlighted how variation in shell growth rhythm could impinge on the spacing between growth halts, the general shape of growth curves and the allometry and ornamentation of the aperture. The structure of shape variation in this population was suggested to mainly result from simple scaling relationships between the aperture dimensions and the lengths of shell segments between successive growth halts.

Such patterns of covariation are then viewed as a reflection of simple growth rules tied to accretionary growth. It is expected that similar patterns of variation may be found in other molluscs, leading to convergent patterns of evolutionary transformations. The covariation between these shells characters seems, at least at first glance, similar to that observed on highly variable ammonoids species (see Fig. 1; to be compared with Figs. 3 & 5 in the introduction chapter). However, the application of the approach developed in this dissertation to the question of the 'Buckman's laws of covariation' in ammonoids requires a better characterization of intraspecific variation in ammonoids. Multivariate statistics are clearly needed to quantify the patterns of covariation between shell characters and to

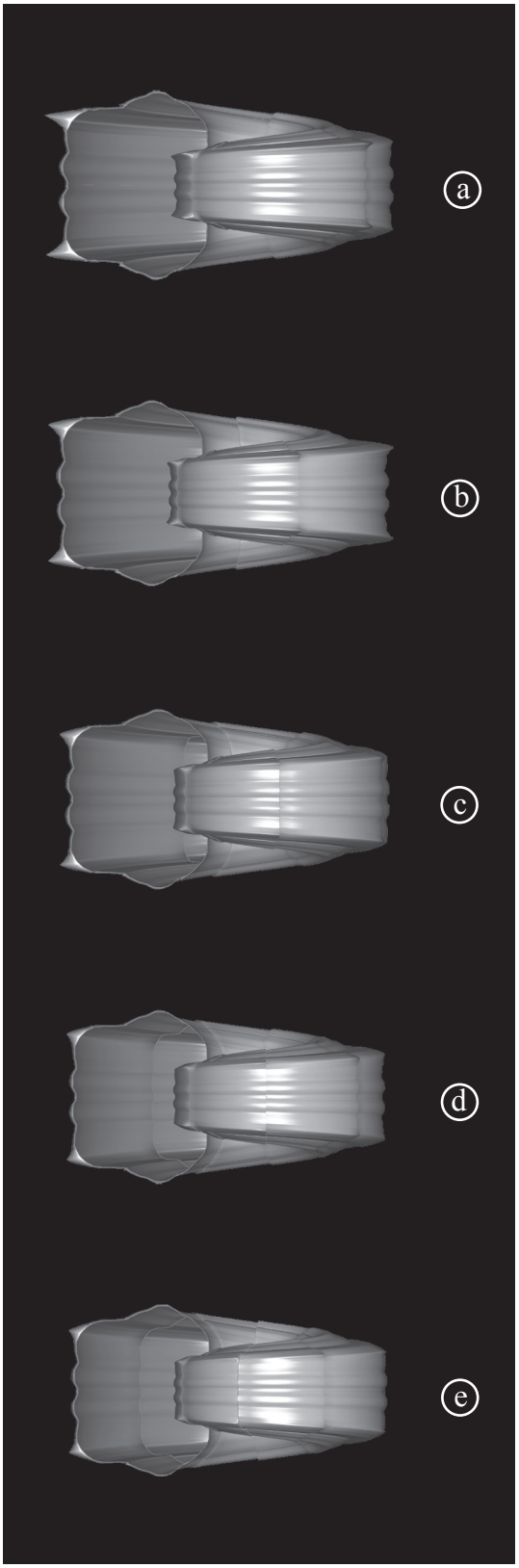


Fig. 1: Simulations of ammonoid-like shells using the growth vector model. See also Fig. 21 of chapter 5. **a-e:** by construction growth halts occur respectively every 16, 14, 12, 10 and 8 growth increments. Note that from top to bottom, shell spininess decreases.

describe ontogenetic allometries. In this thesis, it was pointed out that straightforward relationships between cross-sectional and longitudinal data should not be expected *a priori*. In order to deepen the comparison of patterns of variation between ammonoids and gastropods, we need first to characterize the individual ontogenetic trajectories in ammonoids.

Some technical improvements of the growth vector model would also be of benefit. The simulation of shell thickness and of the decoupling between shell and mantle growth would be necessary to drive further the study of shape and growth variation in intertidal gastropods. Shell surface area should better be computed without neglecting whorl overlap if one wants to apply the model to ammonoid shells. Some new morphometric variables, like involution, should also be introduced in order to facilitate comparison with traditional measurements on ammonoids. Simulation of costulation would be of interest too. The growth vector model could also be linked to mechano-chemical models, to hydrostatics models or to phyletic models to inquire different aspects of shell shape development and evolution.

Also, the growth curves described in *Hexaplex trunculus* could be used as inputs of the growth vector model for further investigations on the possible relationships between growth rates, growth rhythm and allometry.

More generally the framework developed here could assist in formulating and testing new hypotheses of growth of molluscan shells. It paves the way toward the development of data-driven mathematical models which could facilitate the comparison of theoretical and empirical data in the future, and perhaps helps interpreting

them in a developmental, ecological or evolutionary context.

Throughout this dissertation, I argued that the time parameter is mandatory to the study of allometry, if one seeks to understand the relationships between size and shape and how they vary in populations. However, the results have not been interpreted in terms of heterochrony for two reasons:

1- Applying uncritically this concept to the intraspecific level results in a situation whereby almost any change in development can be viewed as resulting from a change in timing.

2- The classical terminology of heterochrony is only meaningful if the growth curves can be linearized by the same mathematical transformation.

In the case study discussed here, applying this terminology would have resulted in a considerable loss of information. Moreover, it has been shown that the shape changes associated with variation in growth curves could be mainly understood in terms of variation in growth rhythm (frequency and amplitude of pulses of growth), regardless of the absolute speed of growth. Further investigations are required to clarify whether or not variation in growth rate does actually play a role in the observed patterns of variation in the herein studied species, and in molluscs in general.

To close up, a quotation by Levins & Lewontin (1980, p. 57) summarizing the mood under which this work has been performed:
"Things are similar: this makes science possible. Things are different: this makes science necessary. At various times in the history of science the important advances have been made either by abstracting away differences to reveal similarity or by emphasizing the richness of variation within a seeming uniformity. But either choice by itself is ultimately misleading. The general does not completely contain the particular as cases; the empiricist refusal to group, generalize, and abstract reduces science to collecting if not specimens then examples. We argue for a strategy which sees the unity of the general and the particular through the explanation of patterns of variation which are themselves higher order generalities that in turn reveal patterns of variation".

ACKNOWLEDGEMENTS

This dissertation is the result of a work initiated in 2000 during my postgraduate studies at the University of Lyon (UCBL, Lyon 1). The project was continued by a ph.D thesis at the University of Zürich (PIMUZ). During these years, I have met many people who helped me more or less directly. I address all my thanks and gratitude to them according to the period over which I made their acquaintance. As some of these people later became my friends, I cannot distinguish between personal and professional contributions. I am in great debt to my thesis advisors and collaborators for their patience, as I struggled to finish the manuscript.

2000: Catching the research virus

The project of this thesis would not have emerged without the concerted expertise of several researchers; namely the initial supervisors of my master thesis: **Hugo Bucher**, **Régis Chirat** and **Stéphane Genieys**.

First of all, I would like to thank my ph.D supervisor, **Hugo Bucher**, who offered me the opportunity to work at the frontier between palaeontology, biology and theoretical modelling. I met Hugo during summer 2000 as I was inquiring myself about the possibility of switching from biological studies to paleontological studies. Our discussion rapidly turned to ammonoids growth and reaction-diffusion models… These matters had fascinated Hugo at least since the end of his own ph.D, and I must admit that his ongoing enthusiasm was infecting. Hugo is sincerely thanked for his open-mind, his support and his never failing confidence in me. He introduced me to climbing (as nearly half of the people in the institute). I enjoyed climbing with Hugo, Thomas Galfetti, Nicolas Goudemand and Christian Klug. Hopefully, I have not let fall anyone of them! *The after-climbing dinners at the 'Oasis' are among my best memories in Zürich, with you guys telling stories about your field works in many far away countries. Often, it made me feel like I had been there.*

Régis Chirat (UCBL, France), who I still consider in many ways as my supervisor, provided thoughtful and critical feedback on my work by playing the 'devil's advocate' (and thus scarcely being of same mind as me). Régis drove me to stand back from things in introducing me to conceptual matters which I first thought were far beyond my reach. His exigent nature, his openness

and friendship have been much appreciated. Of course, Régis is the best spoilsport that I know (it maddened me from time to time). In every instance, he prevented me for taking the easy way out and settled much of the fundamentals of much of my own work. *I did not see you much during these years but your two pages e-mails (at least) compensated, and perhaps improved our communication. Many thanks for the time and energy you devoted in this dissertation.*

Stéphane Genieys (UCBL), and the mathematical lab in Lyon are thanked for introducing me to the world of computation and non-linear differential equations. Because of scientific divergences, this collaboration fell short. The time invested in instructing me about nearly unknown matters is deeply acknowledged.

2002: Arriving to Zürich

Many people helped me in initiating the 'Schnecken Labor'. Without them, the core of this thesis would not have been made possible. Among them, **Sigurd von Boletzky** (Observatoire Océanographique de Banyuls-sur-mer, France) provided the egg capsules and some adult specimens of Muricids. He also gave me a warm welcome during my visit in Banyuls and friendly advices about the beaches and restaurants to go in the surroundings.

Georg Ribi (Zoologisches Museum, Zürich) housed the 'baby snails' in his lab for a couple of months at the begining of this project. He also provided aquarium material. His and **Sonja Sbilordo** (Zoologisches Museum, Zürich)'s advices about aquarium maintenance have been much appreciated.

Øyvind Hammer (Geological Museum of Oslo, Norway) and **Lotte Bank** helped me in initiating the aquarium lab. Lotte's optimism helped me in keeping my calm with the aquariums' filters in the very beginning.

Øyvind Hammer and **Christoph Zollikofer** (Anthropologisches Museum, Zürich) are thanked for their advices on morphometry. **Marcia Ponce de León** (Anthropologisches Museum, Zürich) is thanked for her warm welcome any time I saw her. Øyvind Hammer, Christoph Zollikofer and Marcia Ponce de León's works inspired me and stimulated me in many ways. Although Øyvind kindly provided me a copy of his model of shell growth, I had to entirely rewrite the codes, since I have never been able to find out the right version of the program to open it!

When I arrived to Zürich, several people helped me to feel like home in spite of dialect speaking. Among them, **Ursula Imhof** (secretary, PIMUZ, now retired) took in charge much of the administrative and personal paperwork. She often behaved like a 'mother' toward me (and other students that she used to name 'Die Kinder'), for instance by reminding me to make Xerox of important papers, recalling me the dead lines, advicing me to wear a scarf when it was cold outside, etc...

Heinz Lanz (photographer, PIMUZ, now retired) is thanked for his friendly welcome and his enthusiasm in speaking French with me. Heinz Lanz, **Julia Huber** and **Léonie Pauli** constructed the plastic boxes which were used to breed the snails. These boxes have successfully prevented the newly hatched snails form eating each other. We got lucky!

Markus Hebeisen (Preparator, PIMUZ), provided great help with the aquarium maintenance, particularly during the holidays. In spite of the difficulties in talking together at the beginning, he has always understood what I was inquiring him about and moved heaven and earth to help me (including chemicals detritus to recover my ring fallen in the labour canalizations). Indeed, he learned me most of the german and dialect I know (including the Stichtworten, I have proofs of it!).

Arnaud Brayard (Toulouse) and **Claude Monnet** (PIMUZ) have endured with me the weekly train travels between Zürich and Lyon for about one year. Often, these 6 hours of travel were the occasion of discussions on evolution, and so on.

2003-2006: Institute turn-over: New 'arrivals' in the institute

Jérôme Gapany (librarian, PIMUZ) shared his efficient librarian tips with me from the beginning, so that I was able to find out the most inaccessible papers by myself (well nearly so!). The afterwork beers, at the BQM, Corazòn or Safari bar have provided the occasion of many interesting discussions. Jérôme is thanked for is open-mind and sympathy.

Christian Klug (PIMUZ), famous for his funny but rough jokes, is thanked for his advices, his easy going mind and for having kindly translated my abstract into German.

Thomas Brühwiler (PIMUZ), and later **Carlo Romano** (PIMUZ) were probably the most silent and easy going officemates that one can imagine, never complaining about myself (*well, as far as I know!*).

In the institute, I gained two unconditional friends: **Thomas Galfetti** (PIMUZ) and **Nicolas Goudemand** (PIMUZ). *I will miss you guys…*

During more than 4 years, **Thomas**, my officemate, provided me incredible support, solidarity and friendship. He contributed towards livening things up in the institute and in the office. He introduced me to his passions, photography and minimal electro music. He shared with me many of his tips with Illustrator and helped me with some of the figures of this dissertation (including one that he did twice, *sorry*). *I would not have liked to share my office with someone else. We shared difficult moments, but you were certainly the best "tears and joy companion" that I could have dreamed of*.

The arrival of **Nicolas**, alias Nico, marked a definite turning point in my thesis. Our friendship and collaboration began nearly instantaneously after we met with discussions about automatic detection of morphometric data, pattern recognition and many other things (*you are nearly as talkative as me!*). Nico introduced me to the Matlab world, where his help has been proven decisive. He taught me so many things that I cannot express all my gratitude. In many instances, his optimism convinced me that finishing this ph.D could (perhaps) be possible. Nico is thanked for his many skills to which I have not yet found the limits, his optimism, his support and his talent for understanding my tortuous questions and tiny worries. I acknowledge the time he invested in this work, advising about things going far beyond technical details, turning him into a real collaborator on molluscan development. Nico must be acknowledged for having understood how to derive the 'logarithmic spiral case' from Øyvind Hammer's model; a damned equation that provided me the impetus to develop the rest of this thesis. Nico also mounted nearly all the figure plates of this thesis and endorsed most of the page make-up. Hopefully, he did it with extreme care, so that I have been really pleased with the result and did not have to bother him too much (*isn't it?*) with unaligned things and so on! *I think I have to return you the help for your own ph.D thesis. I promise I will take care of your 500 hundreds plates of photographs of conodonts!*

I enjoyed assisting in teaching with Christian, **Winand Brinkman**, **Peter Hochuli** and **Heinz Furrer**. **Rosi Roth** (photographer, PIMUZ) is thanked for the photographic work.

2006: New arrivals in the institute bis

Andrea Spring (secretary, PIMUZ) has been much appreciated for her full of good-humoured temperament, whatever happens. *Although it is impossible to replace Ursula, I must admit that I deeply regret that you leaved the institute* (*and it is not because you proposed me several times to massage my painful back!*). *Take care of you; your laugh is contagious.*

Marcelo Sánchez-Villagra (PIMUZ) is thanked for having encouraged me to go to the 'Evo-devo' Conference (August 2006) in Prague, where my co-authors and I won the award for the best poster. Your advices have been much appreciated. By the way, **Lennart Olsson** (Institut für Spezielle Zoologie und Evolutionsbiologie, Jena, Germany) is thanked for insightful discussions in the bus toward the last day conference party. Afterwards, he proposed me to write a book review that stimulated me in many ways. I have been flattered to have the occasion of expressing my opinion on cellular automata (something I would not have expected).

There are many people in the institute I did not have the occasion to discuss with, mainly because I have been hidden at home with my work for months. I hope I will have more time now.

The people I have always known (or nearly so)

My parents are thanked for having provided me the emotional and financial support to bring me to my Master Thesis. My parents-in-law are also thanked for always having been ready to help. All my apologize to my family (especially my brother), and my friends, not cited here, that I have not seen much during these years.

Lastly, I gratefully acknowledge **Gilles Beretta**, my lover, for his support, as I doubted I would have the nerve to complete this thesis. *We have been through difficult times, and I apologize for the sacrifices that my moving to Zürich has generated. Thank you for having been waiting for me to finish this thesis.*

Love and kisses,

Séverine

PS: I have forgotten to thank my snails, for not dying before I had finished with them (and that was long). So I cannot deny that snails really grow at a snail's pace!

Curriculum vitae

name	**Séverine Urdy**
date of birth	25.05.1979
nationality	French
place of citizenship	Vienne (Isère, France)
professional address	Paläontologisches Institut und Museum Universität Zürich Karl Schmid Strasse 4 8006 Zürich e-mail < urdy@pim.uzh.ch Ph. +41 44 634 23 45; Fax +41 44 634 49 23

Education

Since 2009	Post-doctoral fellow at the Paleontological Institute of the University of Zürich. Title: *"Growth and form of molluscan shells: experiments and models"*. Swiss National Fond. Grant number: 200021_124784 /1. Advisor: H. Bucher.
2002 - 2008	Ph.D thesis at the Paleontological Institute of the University of Zürich (25th September 2008). Title: *"Generic aspects of molluscan shell morphogenesis: theoretical, experimental and comparative approaches"*. Advisor: H. Bucher.
2000 - 2002	Master's degree of Earth Sciences and Theoretical Biology, University Claude Bernard Lyon 1 (UCBL). Diploma title: *"Simulations of ornamentation in ammonoids"*. Advisors: H. Bucher; R. Chirat & S. Genieys.
1998 - 2000	Bachelor's degree of Life and Earth Sciences, University Claude Bernard Lyon 1 (UCBL).
1997 - 1998	INSA: engineering school in Lyon (first year).
1997	Maturity Degree in Sciences, Academy of Grenoble, France. Highschool: Lycée Saint-Romain-en-Gal, Vienne, Isère, France.

Publications

(1) URDY, S. & CHIRAT, R. (2006). Snail shell coiling (re-)evolution and the evo-devo revolution. *Journal of Zoological Systematics and Evolutionary Research* 44, 1-7.

(2) URDY, S. (2009). Principles of morphogenesis: the contribution of cellular automata models. *Acta Zoologica* 90/2, 205-208. Review of DEUTSCH, A. & DORMANN, S. (2005). Cellular automaton modeling of biological pattern formation: Characterization, Applications, and Analysis. Birkhäuser, Boston, Basel, Berlin.

(3) URDY, S., GOUDEMAND, N., BUCHER, H. & CHIRAT, R. (accepted). Allometries and the morphogenesis of the molluscan shell: a quantitative and theoretical model. *Journal of Experimental Zoology Part B*.

(4) URDY, S., GOUDEMAND, N., BUCHER, H. & CHIRAT, R. (accepted). Growth dependent phenotypic variation of molluscan shell shape: implications for allometric data interpretation. *Journal of Experimental Zoology Part B*.

(5) URDY, S. Evo-devo of morphogenetic models (submitted).

(6) URDY, S., GOUDEMAND, N. & BUCHER, H. Growth dynamics within a population of *Hexaplex trunculus* (Muricidae, Gastropoda) reared in laboratory (submitted).

(7) URDY, S., GOUDEMAND, N. & BUCHER, H. How do recurrent patterns of covaration in molluscan shells relate to growth dynamics? (submitted).

Meeting abstracts

(1) URDY, S., GOUDEMAND, N., BUCHER, H. & CHIRAT, R. (2006). Growth dependent phenotypic variation: a quantitative theoretical approach of molluscan shell morphogenesis. First Meeting of the European Society for Evolutionary Developmental Biology (EED), Prague, Czech Republic. Award for best student poster presentation.

(2) URDY, S., GOUDEMAND, N., BUCHER, H. & MONNET, C. (29th July-1st August 2008). Molluscan shell shape: patterns of variation and growth models. Second Meeting of the European Society for Evolutionary Developmental Biology (EED), Ghent, Belgium. Invited talk.

(3) URDY, S., GOUDEMAND, N., BUCHER, H. & MONNET, C. (5th-8th October 2008). Growth models of recent gastropods shells: implication for ammonoids. The Geological Society of America, Houston, Texas, USA.

Awards

2008 Faculty award for excellent scientific work (Mathematisch-naturwissenschaftliche Fakultät, Universität Zürich).

2006 Best student poster award at the *First Meeting of the European Society for Evolutionary and Developmental Biology*, Prague, Czech Republic.